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(71) Applicant: EURO-CELTIQUE, S.A. [US/US]; P.O. Box HM 2332, 14 Par-La-Ville Road, HMJX, Hamilton (BM).

(72) Inventor: BURCH, Ronald, M.; 12 Powderhorn Hill, Wilton, CT 06897 (US).

(74) Agents: ANTLER, Adriane, M. et al.; Pennie & Edmonds LLP, 1155 Avenue of the Americas, New York, NY 10036 (US).

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(57) Abstract

The invention provides modified immunoglobulin molecules, particularly antibodies, that immunospecifically bind a member of a binding pair which immunoglobulins have a variable domain containing one or more complimentary determining regions that contain the amino acid sequence of a binding site for that member of the binding pair, which site is derived from the other member of the binding pair and is not naturally found in the complementary determining region. The invention further provides for therapeutic and diagnostic use of the modified immunoglobulin.

IMMUNOGLOBULIN MOLECULES HAVING A SYNTHETIC VARIABLE REGION AND MODIFIED SPECIFICITY

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of Provisional application Serial No. 60/065,716, filed November 14, 1997, and Provisional application Serial No. 60/081,403, filed April 10, 1998, both of which are incorporated by reference herein in their entireties.

1. FIELD OF THE INVENTION

The present invention relates to modified immunoglobulin molecules, particularly antibodies, that bind one member of a binding pair and have at least one complementarity determining region (CDR) that contains the amino acid sequence of a binding site for that member of the binding pair. which binding site is derived from the other member of the binding pair. The invention also relates to methods for treating, diagnosing, or screening for diseases and disorders associated with the expression of the member of the binding pair. particularly, cancer or infectious diseases, using the modified antibodies of the invention. The present invention also relates to pharmaceutical compositions and diagnostic kits containing the modified antibodies of the invention.

2. BACKGROUND OF THE INVENTION

2.1. ANTIBODIES AND THE IMMUNE SYSTEM

Antibodies are proteins that belong to the immunoglobulin superfamily. The immunoglobulin superfamily includes T cell receptors, B cell receptors, cell-surface adhesion molecules such as the co-receptors CD4, CD8, CD19, and the invariant domains of the MHC molecules. In their soluble form, antibodies are glycoproteins produced by mature B cells which are also called plasma cells. Antibodies are secreted into the blood and other extracellular fluids to circulate throughout the body in all animals and humans in response to foreign antigens.

Antibodies have two principal functions. The first is to recognize or bind to foreign antigens. The second is to mobilize other elements of the immune system to destroy the foreign entity. The receptors on the surfaces of immune effector cells are designed for recognition of antigens and cell surface markers on other cells. This recognition process imparts information as to whether the markers are self or non-self, and is an important element involved in modulating the immune system response to the presence of antigens.

The portion of an antigen to which an antibody binds is called its antigenic determinant, or epitope. Some antigens are capable of eliciting an immune response, while others are recognized as self by the immune system. Antigens which can elicit an immune response are termed immunogens, and are usually macromolecules of at least 5000 Dalton molecular weight, such as proteins, nucleic acids, carbohydrates, and lipids. Smaller nonimmunogenic molecules, termed haptens, also are capable of stimulating an immune response when coupled to a large carrier molecule.

2.2. STRUCTURE OF ANTIBODIES

The basic complete unit of an antibody is a four-chain Y-shaped structure (Figure 1). In the early 1970s, Wu and Kabat assembled the amino acid sequences of a large collection of antibodies and demonstrated that the structure of antibodies and, in fact, all members of the immunoglobulin superfamily, consists of a constant region and four relatively conserved framework regions of semi-rigid beta-sheet, with three relatively short hypervariable sequence regions known as complementarity determining regions (CDRs) interspersed among them (Wu and Kabat, 1970, *J. Exp. Med.* 132(2):211-250; Wu and Kabat, 1971, *Proc. Natl. Acad. Sci. USA* 68(7):1501-1506). This prediction was confirmed by crystallographic studies of antibody structure (Poljak et al., 1973, *Proc Natl Acad Sci USA* 70(12):3305-3310; Diesenhofer et al., 1976, *Hoppe Seylers Z Physiol Chem* (Germany, West) 357(10):435-445; Diesenhofer et al., 1976, *Hoppe Seylers Z Physiol Chem* (Germany, West) 357(10):1421-1434).

Figure 1 represents the overall structure of an antibody molecule. Antibodies are made up of two shorter light chains linked via disulfide bonds to two longer heavy chains, which are themselves connected by disulfide bonds. As indicated in Figure 2, both the heavy and light antibody protein chains are composed of multiple domains, each about 110 amino acid residues in length. Each light and heavy chain of an antibody has a variable region at its amino terminus (V_L and V_H respectively); it is the variable region of the antibody that confers the antigen-binding specificity. A heavy chain variable domain and a light chain variable domain together form a single antigen-binding site, thus, the basic immunoglobulin unit has two antigen-binding sites.

Diversity in the variable regions of both the light and heavy chains is restricted to the three "hypervariable" regions or CDRs. There are a total of six CDRs in each antibody molecule (Figure 2), each of which CDR contains from about five to about ten amino acids, or up to about 20 amino acids when the CDR is endogenously recombined, as is common in some antibody classes. The three CDRs of the variable region of each light and each heavy

chain form loops which are clustered together and are connected to the four remaining parts of the variable region, called the framework regions ("FRs") which are relatively conserved among antibody molecules. Antibody diversity is generally created by changing the sequences of the CDRs.

The variable regions are distinct for each antibody, whereas the constant regions are more highly conserved. While the light chain has only one constant region domain, the heavy chain constant region is composed of multiple domains, named CH1, CH2, CH3...CHx. The constant region domains are charged with the various antibody effector functions, such as complement binding and binding to the Fc receptors expressed by lymphocytes, granulocytes, monocyte lineage cells, killer the stimulation of B cells to undergo proliferation and cells, mast cells and other immune effector cells. Other effector functions are differentiation, activation of the complement cell lysis system, opsonization, attraction of macrophages. Antibodies of different isotypes have different constant domains and therefore have different effector functions. The best studied isotypes are IgG and IgM.

All animal species express several different classes of antibodies. Five human antibody classes (IgG, IgA, IgM, IgD and IgB), and within these classes, various subclasses, are recognized on the basis of structural differences, such as the number of immunoglobulin units in a single antibody molecule, the disulfide bridge structure of the individual units, and differences in chain length and sequence. IgG antibodies are, thus far, the most generally useful of these classes for diagnostic and therapeutic pharmaceutical uses, although antibodies from other classes may find utility in certain uses.

2.3. ANTIBODY ENGINEERING

The development of monoclonal antibody technology, first disclosed by Kohler and Milstein (1975, *Nature* 256:495-497), has allowed the generation of unlimited quantities of antibodies of precise and reproducible specificity. The Kohler and Milstein procedure involves the fusion of spleen cells obtained from an immunized animal, with an immortal myeloma cell line to produce hybridomas. Clones which produce an antibody having the requisite specificity are then selected from these hybridomas. The hybridomas produce monoclonal antibodies which are uniform in their properties and specificity.

To date, identification and production of suitable antibodies useful in diagnostic and therapeutic applications has depended on chance. The generation of antibody-producing hybridomas involves immunization of a mouse with an antigen, or, alternatively, the antigen is added to spleen cell preparations *in vitro*. The population of spleen cells and, therefore, of

potential monoclonal antibodies with a particular specificity depends upon the animal's immune reaction to the antigen.

Additional approaches to generating antibodies useful for diagnostic and therapeutic uses have been developed as an alternative to the laborious immunization procedure mentioned above. One approach entails the cloning of antibody genes into phage viruses, which will express on the virus surfaces a single variable region as described in Clackson et al., 1991, *Nature* 352:624; Marks et al., 1992, *J. Mol. Biol.* 222:581; Zebedee et al., 1992, *Proc. Natl. Acad. Sci. USA* 39:3175; Gram et al., 1992, *Proc. Natl. Acad. Sci. USA* 89:3576. Using phage library techniques, one can generate large libraries that express much of the inherent genetic diversity. However, such libraries are still constrained by the antibody repertoire from which they were derived. In yet another approach, variable domain genes which are randomly mutagenized and expressed. also result in the production of large libraries as described in Pack (1997, High Quality Antibody Libraries, Abstracts of the Eighth International Conference of Antibody Engineering). While both approaches are successful in generating great diversity, they are generally little more successful in identifying useful antibodies when compared with traditional immunization methods because they rely on random generation of CDR sequences. Moreover, antibodies generated through immunization of mice are of limited use in human therapeutics. Since mouse monoclonal antibodies are foreign and thus immunogenic to humans, they induce a human antimouse antibody (HAMA) response (Shawler et al., 1985, *J. Immunol.* 135:1530; Chatenaud et al., 1986, *J. Immunol.* 137:830).

2.4. PHARMACEUTICALS BASED UPON MANIPULATION OF INTERMOLECULAR INTERACTIONS

The efficacy of a pharmaceutical is often derived from the ability of the pharmaceutical to enhance, antagonize or mimic the binding of one molecule to another, for example, a ligand to its receptor, or a pathogen to a cellular receptor, thereby achieving certain physiological and pharmacological activity useful for disease prevention or amelioration. Until recently, pharmaceuticals were limited to serendipitously discovered synthetic or natural products, and were small molecule effectors that mimicked the binding of naturally occurring ligands. Even when information is available concerning the structure of ligands or their binding sites, currently available methods have not readily led to the development of effective pharmaceuticals. Methods such as the use of molecular modeling to design small molecule analogs based on crystal structure data for ligand-receptor binding pairs, or the screening for binding to a receptor using peptide combinatorial libraries or

natural product extracts, have not proved to be reliable. Additionally, these synthetic or natural products do not always have the ability to discriminate in binding affinity and specificity for receptor subtypes, which can result in undesirable side effects due to insufficient control over the pharmacological effects.

There is a need for a method to more directly reproduce or inhibit the effects of natural interactions, and to be able to design specific pharmaceutical agents that interact with members of a particular binding pair and which more closely mimic the behavior of naturally occurring ligands.

Citation of references hereinabove shall not be construed as an admission that such references are prior art to the present invention.

3. SUMMARY OF THE INVENTION

The present invention is based upon the observation of the present inventors that the binding site contained within one member of a binding pair for another member of the binding pair can be transplanted into at least one CDR of an immunoglobulin molecule to confer specificity on the immunoglobulin for the second member of the binding pair.

The present invention is aimed at providing a method to design, immunoglobulins, particularly antibodies, with a particular specificity, which method circumvents the unpredictable immunization and screening processes currently employed to isolate specific antibodies. In particular, synthetic modified antibodies that immunospecifically bind one member of a binding pair are engineered such that the variable region of the modified antibody has one or more CDRs that contain the binding sequence for that member of the binding pair, which binding sequence is derived from the other member of the binding pair. This method, thus, dramatically simplifies the process of identifying suitable antibodies and makes available antibodies for many antigens that are inaccessible due to immune tolerance or cryptic expression.

Accordingly, the present invention provides modified immunoglobulin molecules, particularly antibodies, that immunospecifically bind a first member of a binding pair, which binding pair consists of the first member and a second member, which antibodies comprise a variable domain which has at least one CDR containing an amino acid sequence of the binding site for the first member of the binding pair, which binding site is derived from the second member of the binding pair. In a preferred aspect of the invention, the amino acid sequence of the binding site is not found naturally within the CDR.

The binding pair can be any two molecules that specifically interact with each other. In specific embodiments, the first member of the binding pair is a cancer antigen (*i.e.*, a molecule expressed on the surface of a cancer cell), an antigen of an infectious disease agent (*i.e.*, a molecule on the surface of an infectious disease agent) or a cellular receptor for an infectious disease agent. Such cancer antigens include human milk fat globule antigen (HMFG), an epitope of polymorphic epithelial mucin antigen (PEM), or a human colon carcinoma-associated protein antigen. Such antigens of infectious disease agents include a Brambell receptor (FcRB), and antigens of HSV-2, gonococcus, Treponema pallidum, Chlamydia trachomatis or human papillomavirus. In other specific embodiments, the binding pair is a receptor-ligand binding pair, for example, where the first member of the binding pair is a bradykinin receptor.

The invention further provides methods of treatment or prevention using the modified immunoglobulins of the invention. For example, modified antibodies having one or more CDRs containing the binding site for a cancer antigen or an antigen of an infectious agent or a cellular receptor for an infectious disease agent can be used in the treatment or prevention of a cancer or an infectious disease associated with the expression of the particular cancer antigen or antigen of the infectious disease agent or the cellular receptor for the infectious disease agent.

The invention further provides methods for screening or detection or diagnosis using the modified immunoglobulins of the invention. For example, modified antibodies having one or more CDRs containing the binding site for a cancer antigen or an antigen of an infectious disease agent can be used in the screening, detection and diagnosis of a cancer or an infectious disease associated with the expression of the particular cancer antigen or antigen of the infectious disease agent.

The invention also provides therapeutic and diagnostic kits and pharmaceutical compositions containing the modified immunoglobulins of the invention.

The invention further provides methods of producing a synthetic modified immunoglobulin of the invention.

Section 6, *infra*, describes the synthesis of synthetic modified antibodies in which one of the CDRs contains an amino acid sequence from bradykinin encompassing the binding sequence for the bradykinin receptor. The example demonstrates that this synthetic modified antibody immunospecifically binds the bradykinin receptor, and competes with bradykinin for binding to the bradykinin receptor. The activity of the synthetic modified antibody is antagonized by an antagonist of bradykinin activity.

4. BRIEF DESCRIPTION OF THE FIGURES

Figure 1. A schematic diagram showing the structure of the light and heavy chains of an immunoglobulin molecule, each chain consisting of a variable region positioned at the amino terminal region (H_2N-) of the immunoglobulin and a constant region positioned at a carboxyl terminal region (-COOH) of the immunoglobulin.

Figure 2. A schematic diagram of an IgG showing the four framework regions (FR1, FR2, FR3 and FR4) and three complementarity determining regions (CDR1, CDR2 and CDR3) in the variable regions of the light and heavy chains (labeled as V_L and V_H respectively). The constant region domains are indicated as C_L for the light chain constant domain and CH_1, CH_2 and CH_3 for the three domains of the heavy chain constant region. Fab indicates the portion of the antibody fragment which includes the variable region domains of both light and heavy chains and the C_L and CH_1 domains. Fc indicates the constant region fragment containing the CH_2 and CH_3 domains.

Figures 3A-C. (A). The structure of the expression vector pMRRO10.1, which contains a human kappa light chain constant region sequence. (B). The structure of the expression vector pGamma1 that contains a sequence encoding a human IgG1 constant region (CH1, CH2, CH3) heavy chain and hinge region sequences. (C) The structure of the expression vector pNEPuDGV which contains a sequence encoding the kappa constant domain of the light chain and the constant domain and hinge region of the heavy chain. For all three vectors see Bebbington et al., 1991, *Methods in Enzymology* 2:136-145.

Figures 4A-H. The amino acid and nucleotide sequences for the heavy and light chain variable domains that have a CDR containing bradykinin sequences and corresponding heavy and light chain variable domain consensus sequences of the synthetic antibodies. All of these sequences also contain a leader sequence. (A) The amino acid sequence and corresponding nucleotide sequence for the consensus light chain variable region ConVL1. (B) The amino acid and corresponding nucleotide sequence for the light chain variable region BKCDR1 in which CDR1 contains a bradykinin sequence. (C) The amino acid and corresponding nucleotide sequences for the light chain variable region BKCDR2 in which CDR2 contains a bradykinin sequence. (D) The amino acid and corresponding nucleotide sequences for the light chain variable region BKCDR3 in which CDR3 contains a bradykinin sequence. (E) The amino acid and corresponding nucleotide sequences for the consensus heavy chain variable region ConVH1. (F) The amino acid and corresponding nucleotide sequences for the heavy chain variable region BKCDR4 in which CDR4 contains a bradykinin sequence. (G) The amino acid and corresponding nucleotide sequences of the

heavy chain variable region BKCDR5 in which the CDR5 contains a bradykinin sequence. (H) The amino acid and corresponding nucleotide sequence of the heavy chain variable region BKCDR6 in which CDR6 contains a bradykinin sequence.

Figure 5. A schematic diagram of the general steps that were followed for assembly of an engineered gene encoding the synthetic modified antibody containing A sequence of bradykinin. The oligonucleotides used to assemble the gene are indicated as "oligo1" to "oligo10".

Figures 6A and B. (A) Nucleotide sequences of the oligonucleotides used to assemble the consensus light chain (ConVL1), and the bradykinin containing light chain variable regions, by the scheme indicated in Figure 5. (B) Nucleotide sequences of the oligonucleotides used to assemble the consensus heavy chain variable region (ConVH1) and the bradykinin containing heavy chain variable regions, as indicated in Figure 5.

Figures 7A-C. (A) Stimulation of PGE_2 synthesis by bradykinin in SV-T2 cells as indicated in ng/well of PGE_2 for each treatment. In the legend below the figure a "-" indicates that cells were incubated in the absence of the factor while "+" indicates that the cells were incubated in the presence of the factor, *i.e.*, either 1 nM bradykinin (upper row) or 1 nM HOE 140, a bradykinin antagonist (lower row). (B) Stimulation of PGE_2 synthesis by certain synthetic modified antibodies having CDRs containing bradykinin sequences is depicted as pg/well PGE_2, as a function of the dilution of the synthetic antibody BKCDR3 (lines with solid squares), BKCDR4 (lines with solid triangles), and BKCDR5 (lines with solid diamonds), the consensus heavy chain variable region (line with solid circles) and media alone (line with open circles). (C) The bar graph depicts PGE_2 stimulation (in PGE_2 in pg/well) in SV-T2 cells incubated in the presence or absence of bradykinin (indicated as "+" or "-", respectively, in legend below graph) and with an antibody having the BKCDR3, BKCDR4, or BKCDR5 variable domain or an antibody having the heavy chain consensus variable domain (ConVH), as indicated above the bars of the graph.

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to modified immunoglobulin molecules, particularly antibodies that immunospecifically bind (*e.g.*, as determined by any method known in the art for determining the binding specificity of an antibody for its antigen, for example, as described in section 5.7, *infra*, and which immunospecific binding excludes non-specific binding, but not necessarily the cross-reactivity often observed with naturally occurring antibodies) a first member of a binding pair and have at least one complementarity determining region (CDR) that contains an amino acid sequence from the second member of

the binding pair, which amino acid sequence is a binding sequence for the first member of the binding pair. The binding pair can be any two molecules, including proteins, nucleic acids, carbohydrates, or lipids, that interact with each other, although preferably the binding partner from which the binding site is derived is a protein molecule. In preferred embodiments, the antibody contains a binding sequence for a cancer antigen (*i.e.*, a molecule on the surface of a cancer or tumor cell), an infectious disease antigen, (*i.e.*, a molecule on the surface of an infectious disease agent), a cellular receptor for a pathogen, or a receptor or ligand (preferably, a receptor or ligand of a receptor-ligand binding pair in which the ligand binds to the receptor and thereby elicits a physiological response).

The present invention also provides for methods of treatment using the modified immunoglobulins of the invention, for example, but not by way of limitation, a modified antibody having at least one CDR containing a binding sequence for a particular cancer antigen or antigen of an infectious disease agent or a cellular receptor for an infectious disease agent can be used to treat or prevent a cancer or an infectious disease characterized by the presence of that particular antigen by binding of the infectious disease agent to the particular receptor.

The present invention also provides for methods of diagnosis and screening using the modified immunoglobulins of the invention, for example, but not by way of limitation, a modified antibody having at least one CDR containing a binding sequence for a particular cancer antigen or antigen of an infectious disease agent can be used to detect a cancer or infectious disease characterized by that particular antigen or by binding of the infectious disease agent to the particular receptor.

For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the subsections which follow.

5.1. MODIFIED IMMUNOGLOBULIN MOLECULES

The invention provides for modified immunoglobulin molecules, particularly antibodies, that immunospecifically bind (*e.g.*, as determined by any method known in the art for determining the binding specificity of an antibody for its antigen, for example, as described in section 5.7, *infra*) to a first member of a binding pair where at least one of the CDRs of the antibody contains a binding site for the first member of the binding pair, which binding site is derived from an amino acid sequence of the other member of the binding pair. In a preferred aspect of the invention, the amino acid sequence of the binding site is not found naturally within the CDR.

The amino acid sequence of the binding site may be identified by any method known in the art. For example, in some instances, the sequence of a member of a binding pair has already been determined to be directly involved in binding the other member of the binding pair. In this case, such a sequence can be used to construct the CDR of a synthetic antibody that specifically recognizes the other member of the binding pair. If the amino acid sequence for the binding site in the one member of the binding pair for the other member of the binding pair is not known, it can be determined by any method known in the art, for example, but not limited to, molecular modeling methods or empirical methods, *e.g.*, by assaying portions (*e.g.*, peptides) of the member for binding to the other member, or by making mutations in the member and determining which mutations prevent binding.

The binding pair can be any two molecules, including proteins, nucleic acids, carbohydrates, or lipids, that interact with each other, although preferably the binding partner from which the binding site is derived is a protein molecule. In preferred embodiments, the modified immunoglobulin contains a binding sequence for a cancer antigen, an infectious disease antigen, a cellular receptor for a pathogen, or a receptor or ligand that participates in a receptor-ligand binding pair.

In specific embodiments, the binding pair is a protein-protein interaction pair which is either homotypic interaction (*i.e.*, is the interaction between two of the same proteins) or a heterotypic interaction (*i.e.*, is the interaction between two different proteins).

In a specific embodiment, the first member is a member of a ligand-receptor binding pair, preferably, of a receptor-ligand binding pair in which the ligand binds to the receptor and thereby elicits a physiological response, such as intracellular signaling. By way of example, and not by way of limitation, the ligand or receptor can be a hormone, autocoid, growth factor, cytokine or neurotransmitter, or receptor for a hormone, autocoid, growth factor, cytokine, or neurotransmitter, or any receptor or ligand involved in signal transduction. (For reviews of signal transduction pathways, see, *e.g* . Campbell, 1997, *J. Pediat.* <u>131</u>:S42-S44; Hamilton, 1997, *J. Leukoc. Biol.* <u>62</u>:145-155; Soede-Bobok & Touw, 1997, *J. Mol. Med.* <u>75</u>:470-477; Heldin, 1995, *Cell* <u>80</u>:213-223; Kishimoto et al., 1994, *Cell* <u>76</u>:253-262; Miyajima et al., 1992, *Annu Rev. Immunol.* <u>10</u>:295-331; and Cantley et al., 1991, *Cell* <u>64</u>:281-302.). In specific embodiments, one member of the binding pair is ligand such as, but not limited to, cholecystokinin, galanin, IL-1, IL-2, IL-4, IL-5, IL-6, IL-11, a chemokine, leptin, a protease, neuropeptide Y, neurokinin-1, neurokinin-2, neurokinin-3, bombesin, gastrin, corticotropin releasing hormone, endothelin, melatonin, somatostatin, vasoactive intestinal peptide, epidermal growth factor, tumor necrosis factor, dopamine, endothelin, or a receptor for any of these ligands. In other embodiments, one member of the

binding pair is a receptor, such as, but not limited to, an opioid receptor, a glucose transporter, a glutamate receptor, an orphanin receptor, erythropoietin receptor, insulin receptor, tyrosine kinase (TK)-receptor, KIT stem cell factor receptor, nerve growth factor receptor, insulin-like growth factor receptor, granulocyte-colony stimulating factor receptor, somatotropin receptor, glial-derived neurotrophic factor receptor or gp39 receptor, G-protein receptor class or ß2-adrenergic receptor, or a ligand that binds any of these receptors. In another embodiment, one of the members of the binding pair is a ligand gated ion channel, such as but not limited to a calcium channel, a sodium channel, or a potassium channel. In certain embodiments, the invention provides modified immunoglobulins that immunospecifically bind a receptor and are antagonists the ligand that binds that receptor, for example, but not by way of limitation, are antagonists of endorphin, enkephalin or nociceptin. In other embodiments, the invention provides synthetic modified antibodies that immunospecifically bind a receptor and are agonists of the receptor, for example, but not by way of limitation, the endorphin, enkephalin, or nociceptin receptors. In a preferred embodiment, the modified immunoglobulin does not bind the fibronectin receptor. In another preferred embodiment, the binding sequence is not Arg-Gly-Asp, is not a multimer of a binding sequence, and preferably is not a multimer of the sequence Arg-Gly-Asp.

In other specific embodiments, the modified immunoglobulin has a CDR that contains a binding site for a transcription factor. In a preferred aspect, the modified immunoglobulin does not bind to a specific DNA sequence, particularly does not bind to a transcription factor binding site.

In preferred embodiments, the modified immunoglobulin has at least one CDR that contains an amino acid sequence of a binding site for a cancer antigen or a tumor antigen (*e.g.*, as described in detail in section 5.3.1, *infra*.); more preferably the antigen is human colon carcinoma-associated antigen or epithelial mucin antigen. In other embodiments, at least one CDR of the modified immunoglobulin contains an amino acid sequence for a binding site for a human milk fat globule receptor. In other embodiments, the modified immunoglobulin has at least one CDR that contains an amino acid sequence of a binding site for an antigen of a tumor of the breast, ovary, uterus, prostate, bladder, lung, skin, pancreas, colon, gastrointestinal tract, B lymphocytes, or T lymphocytes.

In other preferred embodiments of the invention, at least one CDR of the modified antibody contains an amino acid sequence for a binding site for an antigen of an infectious disease agent (*e.g.*, as described in detail in section 5.3.2, *infra*.), or a binding site for a cellular receptor of an infectious disease agent, preferably where the binding site is not an amino acid sequence of a *Plasmodium* antigen, or is not the binding site Asn-Ala-Asn-Pro or

Asn-Val-Asp-Pro. In additional embodiments, the modified antibody has a CDR that contains the binding site for a bacterial or viral enzyme.

The modified immunoglobulin molecules of the inventin can be derived from any type of immunoglobulin molecule, for example, but not limited to, antibodies, T cell receptors, B cell receptors, cell-surface adhesion molecules such as the co-receptors CD4, CD8, CD19, and the invariant domains of MHC molecules. In a preferred embodiment of the invention, the modified immunoglobulin molecule is an antibody, which can be any class of antibody, *e.g.*, an IgG, IgE, IgM, IgD or IgA, preferably, the antibody is an IgG. In addition the antibody many be of any subclass of the particular class of antibodies. In another specific embodiment, the modified immunoglobulin molecule is a T cell receptor.

The immunoglobulin which is modified to generate the modified immunoglobulin can be any available immunoglobulin molecule and is preferably a monoclonal antibody or is a synthetic antibody. The antibody that is modified may be a naturally occurring or previously existing antibody or may be synthesized from known antibody consensus sequences, such as the consensus sequences for the light and heavy chain variable regions in Figures 4A and B, or any other antibody consensus or germline (*i.e.*, unrecombined genomic sequences) sequences (*e.g.*, those antibody consensus and germline sequences described in Kabat et al., 1991, Sequences of Proteins of Immunological Interest, 5th edition, NIH Publication No. 91-3242, pp 2147-2172).

As noted *supra*, each antibody molecule has six CDR sequences, three on the light chain and three on the heavy chain, and five of these CDRs are germline CDRs (*i.e.*, are directly derived from the germline genomic sequence of the animal, without any recombination) and one of the CDRs is a non-germline CDR (*i.e.*, differs in sequence from the germline genomic sequence of the animal and is generated by recombination of the germline sequences). Whether a CDR is a germline or non-germline sequence can be determined by sequencing the CDR and then comparing the sequence with known germline sequences, *e.g.*, as listed in Kabat et al. (1991, Sequences of Proteins of Immunological Interest, 5th edition, NIH Publication No. 91-3242, pp 2147-2172). Significant variation from the known germline sequences indicates that the CDR is a non-germline CDR.

Accordingly, in other embodiments of the invention, the CDR that contains the amino acid sequence of the binding site is a germline CDR or, alternatively, is a non-germline CDR.

The binding site can be inserted into any of the CDRs of the antibody, and it is within the skill in the art to insert the binding site into different CDRs of the antibody and then screen the resulting modified antibodies for the ability to bind to the particular member

of the binding pair, *e.g.* as discussed in Section 5.7, *infra*. Thus, one can determine which CDR optimally contains the binding site. In specific embodiments, a CDR of either the heavy or light chain variable region is modified to contain the amino acid sequence of the binding site. In another specific embodiment, the modified antibody contains a variable domain in which the first, second or third CDR of the heavy variable region or the first, second or third CDR of the light chain variable region contains the amino acid sequence of the binding site. In another embodiment of the invention, more than one CDR contains the amino acid sequence of the binding site or more than one CDR each contains a different binding site for the same molecule or contains a different binding site for a different molecule. In particular, embodiments, two, three, four, five or six CDRs have been engineered to contain a binding site for the first member of the binding pair. In a preferred embodiment, one or more CDRs contain a binding site for the first member of a binding pair and one or more other CDRs contain a binding site for a molecule on the surface of an immune cell, such as, but not limited to, a T cell, B cell, NK cell, K cell, TIL cell or neutrophil. For example, a modified antibody having a binding site for a cancer antigen or an infectious disease antigen and a binding site for a molecule on the surface of an immune cell can be used to target the immune cell to a cancer cell bearing the cancer antigen or to the infectious disease agent.

In specific embodiments of the invention, the binding site amino acid sequence is either inserted into the CDR without replacing any of the amino acid sequence of the CDR itself or, alternatively, the binding site amino acid sequence replaces all or a portion of the amino acid sequence of the CDR. In specific embodiments, the binding site amino acid sequence replaces 1, 2, 5, 8, 10, 15, or 20 amino acids of the CDR sequence.

The amino acid sequence of the binding site present in the CDR can be the minimal binding site necessary for the binding of the member of the binding pair (which can be determined empirically by any method known in the art); alternatively, the binding site can be greater than the minimal binding site necessary for the binding of the member of the binding pair. In particular embodiments, the binding site amino acid sequence is at least 4 amino acids in length, or is at least 6, 8, 10, 15, or 20 amino acids in length. In other embodiments the binding site amino acid sequence is no more than 10, 15, 20, or 25 amino acids in length, or is 5-10, 5-15, 5-20, 10-15, 10-20 or 10-25 amino acids in length.

In addition, the total length of the CDR (*i.e.*, the combined length of the binding site sequence and the rest of the CDR sequence) should be of an appropriate number of amino acids to allow binding of the antibody to the antigen. CDRs have been observed to have a

range of numbers of amino acid residues, and the observed size ranges for the CDRs (as denoted by the abbreviations indicated in figure 2) are provided in Table 1.

Table 1

CDR	Number of residues
L1	10-17
L2	7
L3	7-11
H1	5-7
H2	9-12
H3	2-25

(compiled from data in Kabat and Wu, 1971, *Ann. NY Acad. Sci.* 190:382-93)

While many CDR H3 regions are of 5-9 residue in length, certain CDR H3 regions have been observed that are much longer. In particular, a number of antiviral antibodies have heavy chain CDR H3 regions of 17-24 residues in length.

Accordingly, in specific embodiments of the invention, the CDR containing the binding site is within the size range provided for that particular CDR in Table 1, *i.e.*, if it is the first CDR of the light chain, L1, the CDR is 10 to 17 amino acid residues; if it is the second CDR of the light chain, L2, the CDR is 7 amino acid residues; if it is the third CDR of the light chain, L3, the CDR is 7 to 11 amino acid residues; if it is the first CDR of the heavy chain, H1, the CDR is 5 to 7 amino acid residues; if it is the second CDR of the heavy chain, H2, the CDR is 9 to 12 amino acid residues; and if it is the third CDR of the heavy chain, H3, the CDR is 2 to 25 amino acid residues. In other specific embodiments, the CDR containing the binding site is 5-10, 5-15, 5-20, 11-15, 11-20, 11-25, or 16-25 amino acids in length. In other embodiments, the CDR containing the binding site is at least 5, 10, 15, or 20 amino acids or is no more than 10, 15, 20, 25, or 30 amino acids in length.

In specific embodiments the modified immunoglobulin of the invention contains a portion of a variable region, *i.e.*, where either the heavy or the light chain contains less than the framework regions and three CDRs, for example but not limited to, where the variable region contains one or two CDRs, and preferably, the intervening framework regions.

In a specific embodiment, the modified antibody immunospecifically binds the bradykinin receptor (for example, but not limited to the modified antibody described in section 6, *infra*). In particular, the embodiment provides a modified antibody in which at

least one CDR contains the amino acid sequence Arg-Pro-Pro-Gly-Phe-Gly-Phe-Ser-Pro-Phe-Arg.

In other specific embodiments, the modified antibody immunospecifically binds the human milk fat globule antigen, and at least one of the CDRs of the modified antibody contains an amino acid sequence selected from the following: (i) Ala-Tyr-Trp-Ile-Glu; (ii) Glu-Ile-Leu-Pro-Gly-Ser-Asn-Asn-Ser-Arg-Tyr-Asn-Glu-Lys-Phe-Lys-Gly; (iii) Ser-Glu-Asp-Ser-Ala-Val-Tyr-Tyr-Cys-Ser-Arg-Ser-Tyr-Asp-Phe-Ala-Trp-Phe-Ala-Tyr; (iv) Lys-Ser-Ser-Gln-Ser-Leu-Leu-Tyr-Ser-Ser-Asn-Gln-Lys-Ile-Tyr-Leu-Ala; (v) Trp-Ala-Ser-Thr-Arg-Glu-Ser; and (vi) Gln-Gln-Tyr-Tyr-Arg-Tyr-Pro-Arg-Thr.

In a more specific embodiment, the CDRs of the heavy chain variable region contain the amino acid sequences (i)-(iii) above, whereas the CDRs of the light chain variable region contain the amino acid sequences (iv)-(vi) above.

In specific embodiments, the invention provide a modified antibody that binds human colon carcinoma-associated antigen and comprises a variable region having at least one CDR containing one of the following amino acid sequences: Thr-Ala-Lys-Ala-Ser-Gln-Ser-Val-Ser-Asn-Asp-Val-Ala; Ile-Tyr-Tyr-Ala-Ser-Asn-Arg-Tyr-Thr; Phe-Ala-Gln-Gln-Asp-Tyr-Ser-Ser-Pro-Leu-Thr; Phe-Thr-Asn-Tyr-Gly-Met-Asn; Ala-Gly-Trp-Ile-Asn-Thr-Tyr-Thr-Gly-Glu-Pro-Thr-Tyr-Ala-Asp-Asp-Phe-Lys-Gly; or Ala-Arg-Ala-Tyr-Tyr-Gly-Lys-Tyr-Phe-Asp-Tyr.

After constructing antibodies containing modified CDRs, the modified antibodies can be further altered and screened to select an antibody having higher affinity or specificity. Antibodies having higher affinity or specificity for the target antigen may be generated and selected by any method known in the art. For example, but not by way of limitation, the nucleic acid encoding the synthetic modified antibody can be mutagenized either randomly, *i.e.*, by chemical or site-directed mutagenesis, or by making particular mutations at specific positions in the nucleic acid encoding the modified antibody, and then screening the antibodies exposed from the mutated nucleic acid molecules for binding affinity for the target antigen. Screening can be accomplished by testing the expressed antibody molecules individually or by screening a library of the mutated sequences, *e.g.*, by phage display techniques (see, *e.g.*, U. S. Patent Nos. 5,223,409; 5,403,484; and 5,571,698, all by Ladner et al; PCT Publication WO 92/01047 by McCafferty et al. or any other phage display technique known in the art).

Accordingly, in a specific embodiment, the modified antibody has a higher specificity or affinity for an antigen than a naturally occurring antibody that

immunospecifically binds the same antigen. In another embodiment, the modified antibody exhibits a binding constant for an antigen of at least $2x10^7$ M.

The modified antibodies of the invention may also be further modified in any way know in the art for the modification of antibodies as long as the further modification does not prevent or inhibit binding of the modified antibody to the particular antigen. In particular, the modified antibodies of the invention may have one or more amino acid substitutions, deletions, insertions besides the insertion into or replacement of CDR sequences with the amino acid sequence of a binding sequence. Such amino acid substitutions, deletions or insertions can be any substitution, deletion or insertion that does not prevent or inhibit the immunospecific binding of the modified antibody to the target antigen. For example, such amino acid substitutions include substitutions of functionally equivalent amino acid residues. For example, one or more amino acid residues can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

Additionally, one or more amino acid residues within the sequence can be substituted by a nonclassical amino acid or chemical amino acid analogs can be introduced as a substitution or addition into the immunoglobulin sequence. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids, α-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, γ-Abu, ε-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, fluoro-amino acids, designer amino acids such as β-methyl amino acids, Cα-methyl amino acids, Nα-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

In a particular embodiment of the invention, the modified immunoglobulin has been further modified to enhance its ability to elicit an anti-idiotype response, for example, as described in co-pending United States Patent Application Serial No. _____, entitled "Modified Antibodies with Enhanced Ability to Elicit An Anti-Idiotype Response" by Burch, filed November 13, 1998 (attorney docket no. 6750-015), which is incorporated by

reference herein in its entirety. Such modifications are made to reduce the conformational constraints on a variable region of the immunoglobulin, *e.g.*, by removing or reducing intrachain or interchain disulfide bonds. Specifically, the modified immunoglobulin is further modified such that one or more variable region cysteine residues that form disulfide bonds are replaced with an amino acid residue that does not have a sulfhydryl group.

Identifying the cysteine residues that form a disulfide bond in a variable region of a particular antibody can be accomplished by any method known in the art. For example, but not by way of limitation, it is well known in the art that the cysteine residues that form intrachain disulfide bonds are highly conserved among antibody classes and across species. Thus, the cysteine residues that participate in disulfide bond formation can be identified by sequence comparison with other antibody molecules in which it is known which residues form a disulfide bond (for example the consensus sequences provided in Figures 4 A and E, or those described in Kabat et al, 1991, sequences of Proteins of Immunological Interest, 5th Ed., U.S. Department of Health and Human Services, Bethesda, Maryland).

Table 2 provides a list of the positions of disulfide bond forming cysteine residues for a number of antibody molecules.

Table 2

(derived from Kabat et al, 1991, sequences of Proteins of Immunological Interest, 5th Ed., U.S. Department of Health and Human Services, Bethesda, Maryland).

Species	Variable domain	Subgroup	Disulfide bond-forming cysteines (positions)
Human	kappa light	I	23,88
Human	kappa light	II	23,88
Human	kappa light	III	23,88
Human	kappa light	IV	23,88
Human	lambda light	I	23,88
Human	lambda light	II	23,88
Human	lambda light	IIII	23,88
Human	lambda light	IV	23,88
Human	lambda light	V	23,88
Human	lambda light	VI	23,88
Mouse	kappa light	I	23,88
Mouse	kappa light	II	23,88
Mouse	kappa light	III	23,88
Mouse	kappa light	IV	23,88

Species	Variable domain	Subgroup	Disulfide bond-forming cysteines (positions)
Mouse	kappa light	V	23,88
Mouse	kappa light	VI	23,88
Mouse	kappa light	VII	23,88
Mouse	kappa light	Miscellaneous	23,88
Mouse	lambda light		23,88
Chimpanzee	lambda light		23,88
Rat	kappa light		23,88
Rat	lambda light		23,88
Rabbit	kappa light		23,88
Rabbit	lambda light		23,88
Dog	kappa light		23,88
Pig	kappa light		23 (88)
Pig	lambda light		23,88
Guinea pig	lambda light		23 (88)
Sheep	lambda light		23,88
Chicken	lambda light		23,88
Turkey	lambda light		23 (88)
Ratfish	lambda light		23 (88)
Shark	kappa light		23,88
Human	heavy	I	22,92
Human	heavy	II	22,92
Human	heavy	III	22,92
Mouse	heavy	I (A)	22,92
Mouse	heavy	I (B)	22,92
Mouse	heavy	II (A)	22,92
Mouse	heavy	II (B)	22,92
Mouse	heavy	II (C)	22,92
Mouse	heavy	III (A)	22,92
Mouse	heavy	III(B)	22,92
Mouse	heavy	III (C)	22,92
Mouse	heavy	III (D)	22,92
Mouse	heavy	V (A)	22,92
Mouse	heavy	V (B)	22,92
Mouse	heavy	Miscellaneous	22,92
Rat	heavy		22,92
Rabbit	heavy		22,92
Guinea pig	heavy		22,92
Cat	heavy		22 (92)
Dog	heavy		22,92
Pig	heavy		22 (92)
Mink	heavy		22 (92)
Sea lion	heavy		22 (92)
Seal	heavy		22 (92)
Chicken	heavy		22,92

Species	Variable domain	Subgroup	Disulfide bond-forming cysteines (positions)
Duck	heavy		22 (92)
Goose	heavy		22 (92)
Pigeon	heavy		22 (92)
Turkey	heavy		22 (92)
Caiman	heavy		22, 92
Xenopus frog	heavy		22,92
Elops	heavy		22,92
Goldfish	heavy		22,92
Ratfish	heavy		22 (92)
Shark	heavy		22,92

Position numbers enclosed by () indicate that the protein was not sequenced to that position, but the residue is inferred by comparison to known sequences.

Notably, for all of the antibody molecules listed in Table 2, the cysteine residues that form the intrachain disulfide bonds are residues at positions 23 and 88 of the light chain variable domain and residues at positions 22 and 92 of the heavy chain variable domain. The position numbers refer to the residue corresponding to that residue in the consensus sequences as defined in Kabat, (1991, Sequences of Proteins of Immunological Interest, 5th Ed., U.S. Department of Health and Human Services, Bethesda, Maryland) or as indicated in the heavy and light chain variable region sequences depicted in Figures 4A and E, respectively (as determined by aligning the particular antibody sequence with the consensus sequence or the heavy or light chain variable region sequence depicted in Figures 4A and E).

Accordingly, in one embodiment of the invention, the modified immunoglobulin molecule is further modified such that the residues at positions 23 and/or 88 of the light chain are substituted with an amino acid residue that does not contain a sulfhydryl group and/or the residues at positions 22 and/or 92 are substituted with an amino acid residue that does not contain a sulfhydryl group.

The amino acid residue that substitutes for the disulfide bond forming cysteine residue is any amino acid residue that does not contain a sulfhydryl group, *e.g.*, alanine, arginine, asparagine, aspartate (or aspartic acid), glutamine, glutamate (or glutamic acid), glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine or valine. In a preferred embodiment, the cysteine residue is replaced

with a glycine, serine, threonine, tyrosine, asparagine, or glutamine residue, most preferably with an alanine residue.

Additionally, the disulfide bond forming cysteine residue may be replaced by a nonclassical amino acid or chemical amino acid analog, such as those listed *supra*, that does not contain a sulfhydryl group.

In specific embodiments, the substitution of the disulfide bond forming residue is in the heavy chain variable region or is in the light chain variable region or is in both the heavy chain and light chain variable regions. In other specific embodiments, one of the residues that forms a particular disulfide bond is replaced (or deleted) or, alternatively, both residues that form a particular disulfide bond may be replaced (or deleted).

In other specific embodiments, the invention provides functionally active fragments of a modified immunoglobulin. Functionally active fragment means that the fragment can immunospecifically bind the target antigen as determined by any method known in the art to determine immunospecific binding (*e.g.*, as described in Section 5.7 *infra*). For example, such fragments include but are not limited to: $F(ab')_2$ fragments, which contain the variable regions of both the heavy and the light chains, the light constant region and the CH1 domain of the heavy chain, which fragments can be generated by pepsin digestion of the antibody, and the Fab fragments, generated by reducing the disulfide bonds of an $F(ab')_2$ fragment (Figure 1; King et., 1992, *Biochem. J.* 281:317); and Fv fragments, *i.e.*, fragments that contain the variable region domains of both the heavy and light chains (Reichmann and Winter, 1988, *J. Mol. Biol.* 203:825; King et al., 1993, *Biochem. J.* 290:723).

The invention also includes single chain antibodies (SCA) (U.S. Patent 4,946,778; Bird. 1988, *Science* 242:423-426; Huston et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-5883; and Ward et al., 1989, *Nature* 334:544-546). Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Additionally, the invention also provides heavy chain and light chain dimers and diabodies.

The invention further provides modified antibodies that are also chimeric or humanized antibodies. A chimeric antibody is a molecule in which different portions of the antibody molecule are derived from different animal species, such as those having a variable region derived from a murine mAb and a constant region derived from a human immunoglobulin constant region, *e.g.*, humanized antibodies. Techniques have been developed for the production of chimeric antibodies (Morrison et al., 1984, *Proc. Natl. Acad. Sci.* 81:6851-6855; Neuberger et al., 1984, *Nature* 312:604-608; Takeda et al., 1985, *Nature* 314:452-454; International Patent Application No. PCT/GB85/00392 (Neuberger et

al. and Celltech Limited)) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. In a specific embodiment, the synthetic modified antibody is a chimeric antibody containing the variable domain of a non-human antibody and the constant domain of a human antibody.

In a more preferred embodiment, the modified antibody is a humanized antibody, particularly an antibody in which the CDRs of the antibody (except for the one or more CDRs containing the binding sequence) are derived from an antibody of a non-human animal and the framework regions and constant region are from a human antibody (U.S. Patent No. 5,225,539 by Winter). Such CDR-grafted antibodies have been successfully constructed against various antigens, for example, antibodies against IL-2 receptor as described in Queen et al., 1989, *Proc. Natl. Acad. Sci USA* 86:10029; antibodies against cell surface receptors-CAMPATH as described in Riechmann et al., 1988, *Nature* 332:323; antibodies against hepatitis B in Co et al., 1991, *Proc. Natl. Acad. Sci USA* 88:2869; as well as against viral antigens of the respiratory syncitial virus in Tempest et al., 1991, *Bio-Technology* 9:267.

CDR-grafted variable region genes have been constructed by various methods such as site-directed mutagenesis as described in Jones et al., 1986, *Nature* 321:522; Riechmann et al., 1988, *Nature* 332:323; *in vitro* assembly of entire CDR-grafted variable regions (Queen et al., 1989, *Proc. Natl. Acad. Sci. USA* 86:10029); and the use of PCR to synthesize CDR-grafted genes (Daugherty et al., 1991, *Nucleic Acids Res.* 19:2471). CDR-grafted antibodies are generated in which the CDRs of the murine monoclonal antibody are grafted onto the framework regions of a human antibody. Following grafting, most antibodies benefit from additional amino acid changes in the framework region to maintain affinity, presumably because framework residues are necessary to maintain CDR conformation, and some framework residues have been demonstrated to be part of the antigen combining site. Thus, in specific embodiments of the invention, the modified antibody comprises a variable domain in which at least one of the framework regions has one or more amino acid residues that differ from the residue at that position in the naturally occurring framework region.

In a preferred embodiment of the invention, the modified antibody is derived from a human monoclonal antibody. The creation of completely human monoclonal antibodies is possible through the use of transgenic mice. Transgenic mice in which the mouse immunoglobulin gene loci have been replaced with human immunoglobulin loci provide *in vivo* affinity-maturation machinery for the production of human immunoglobulins.

In certain embodiments, the modified immunoglobulin (or fragment thereof) is fused via a covalent bond (for example, but not by way of limitation, a peptide bond) at either the N-terminus or the C-terminus to an amino acid sequence of another protein (or portion thereof, preferably an at least 10, 20, or 50 amino acid portion thereof) that is not the modified immunoglobulin. Preferably, the modified immunoglobulin is covalently linked to the other protein at the N-terminus of the constant domain of the modified immunoglobulin. In preferred embodiments, the invention provides fusion proteins in which the modified immunoglobulin is covalently linked to a portion of a growth enhancing factor or; a portion of an immunostimulatory factor, including interleukin-2, interleukin-4, interleukin-5, interleukin-6, interleukin-7, interleukin-10, interleukin-12, interleukin-15, G-colony stimulating factor, tumor necrosis factor, poiin, interferon-gamma, and NK cell antigen or MHC derived peptide.

The modified immunoglobulin may be further modified, *e.g*, by the covalent attachment of any type of molecule, as long as such covalent attachment does not prevent or inhibit immunospecific binding of the immunoglobulin to its target antigen. For example, but not by way of limitation, the modified immunoglobulin may be further modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the modified antibody may contain one or more non-classical amino acids, *e.g.*, as listed above in this Section.

In specific embodiments of the invention, the modified immunoglobulin (or a fragment thereof) is covalently linked to a therapeutic molecule, for example, to target the therapeutic molecule to a particular cell type or tissue, *e.g.*, a cancer or tumor cell. The therapeutic molecule can be any type of therapeutic molecule known in the art, for example, but not limited to, a chemotherapeutic agent, a toxin, such as ricin, an antisense oligonucleotide, a radionuclide, an antibiotic, anti-viral, or anti-parasitic, etc.

5.2. METHODS OF PRODUCING THE MODIFIED IMMUNOGLOBULIN

The modified immunoglobulins of this invention can be produced by any method known in the art for the synthesis of immunoglobulins, in particular, by chemical synthesis or by recombinant expression, and is preferably produced by recombinant expression techniques.

Recombinant expression of the modified immunoglobulin of the invention, or fragment thereof, requires construction of a nucleic acid encoding the modified immunoglobulin. Such an isolated nucleic acid which contains a nucleotide sequence encoding the modified immunoglobulin can be produced using any method known in the art, for example, recombinant techniques or chemical synthesis (*e.g.*, see Creighton, 1983, "Proteins: Structures and Molecular Principles", W.H. Freeman & Co., N.Y.. pp.34-49; and Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual. Cold Springs Harbor Press, N.Y.), or using PCR on known immunoglobulin genes to engineer the nucleotide sequence encoding the CDR sequence containing the binding site.

Accordingly, the invention provides nucleic acids that contain a nucleotide sequence encoding a modified immunoglobulin of the invention, or a functionally active fragment thereof.

Preferably, a nucleic acid that encodes a modified immunoglobulin may be assembled from chemically synthesized oligonucleotides (*e.g.*, as described in Kutmeier et al., 1994, Biotechniques 17:242), which, briefly, involves the synthesis of a set of overlapping oligonucleotides containing portions of the sequence encoding the modified immunoglobulin, annealing and ligation of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR, *e.g.*, as exemplified in Section 6, *infra*.

Accordingly, the invention provides a method of producing a nucleic acid encoding a modified immunoglobulin, said method comprising: (a) synthesizing a set of oligonucleotides, said set comprising oligonucleotides containing a portion of the nucleotide sequence that encodes the synthetic modified immunoglobulin and oligonucleotides containing a portion of the nucleotide sequence that is complementary to the nucleotide sequence that encodes the synthetic modified immunoglobulin, and each of said oligonucleotides having overlapping terminal sequences with another oligonucleotide of said set, except for those oligonucleotides containing the nucleotide sequences encoding the N-terminal and C-terminal portions of the synthetic modified immunoglobulin; (b) allowing the oligonucleotides to hybridize or anneal to each other; and (c) ligating the hybridized

oligonucleotides, such that a nucleic acid containing the nucleotide sequence encoding the synthetic modified immunoglobulin is produced.

Alternatively, a nucleic acid containing a nucleotide sequence encoding a modified immunoglobulin can be constructed from a nucleic acid containing a nucleotide sequence encoding, *e.g.*, an antibody molecule, or at least a variable region of an antibody molecule. Nucleic acids containing nucleotide sequences encoding antibody molecules can be obtained either from existing clones of antibody molecules or variable domains or by isolating a nucleic acid encoding an antibody molecule or variable domain from a suitable source, preferably a cDNA library *e.g.*, an antibody DNA library or a cDNA library prepared from cells or tissue expressing a repertoire of antibody molecules or a synthetic antibody library (see, *e.g.*, Clackson et al., 1991, *Nature* 352:624; Hane et al., 1997, *Proc. Natl. Acad. Sci USA* 94:4937), for example, by hybridization using a probe specific for the particular antibody molecule or by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence.

Once a nucleic acid containing a nucleotide sequence encoding at least a variable region of an antibody molecule has been cloned, then the binding site sequence can be inserted into the nucleotide sequence coding for one or more of the CDRs. Such engineering of the particular CDR coding sequence can be accomplished by routine recombinant DNA techniques known in the art. For example, the nucleotide sequence encoding the CDR can be replaced by a nucleotide sequence encoding the CDR containing the particular binding site sequence, for example, using PCR based methods, *in vitro* site directed mutagenesis, etc. If a convenient restriction enzyme site is available in the nucleotide sequence of the CDR, then the sequence can be cleaved with the restriction enzyme and a nucleic acid fragment containing the nucleotide sequence encoding the binding site can be ligated into the restriction site. The nucleic acid fragment containing the binding site can be obtained either from a nucleic acid encoding all or a portion of the protein containing the binding site or can be generated from synthetic oligonucleotides containing the sequence encoding the binding site and its reverse complement.

The nucleic acid encoding the modified antibody optionally contains a nucleotide sequence encoding a leader sequence that directs the secretion of the synthetic modified antibody molecule.

Once a nucleic acid encoding at least the variable domain of the modified antibody is obtained, it may be introduced into a vector containing the nucleotide sequence encoding the constant region of the antibody (see, *e.g.*, PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464). Vectors containing the complete light or

heavy chain for co-expression with the nucleic acid to allow the expression of a complete antibody molecule are also available and are known in the art, for example, pMRRO10.1 and pGamma1 (see also Bebbington, 1991, *Methods in Enzymology* 2:136-145).

The expression vector can then be transferred to a host cell by conventional techniques and the transfected cells can be cultured by conventional techniques to produce the antibody of the invention. Specifically, once a nucleic variable region of the modified antibody has been generated, the modified antibody can be expressed, for example, by the method exemplified in Section 6. (See also Bebbington, 1991, *Methods in Enzymology* 2:136-145.) For example, by transient transfection of the expression vector encoding the modified immunoglobulin into COS cells, culturing the cells for an appropriate period of time to permit immunoglobulin expression, and then taking the supernatant from the COS cells, which supernatant contains the secreted, expressed modified immunoglobulin.

The host cells used to express the recombinant antibody of the invention may be either bacterial cells such as *Escherichia coli*, particularly for the expression of recombinant antibody fragments or, preferably, eukaryotic cells, particularly for the expression of recombinant antibody molecules. In particular, mammalian cells such as Chinese hamster ovary cells (CHO) or COS cells, used in conjunction with a vector in which expression of the antibody is under control of the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for immunoglobulins (Foecking et al., 1986, *Gene* 45:101; Cockett et al., 1990, *Bio/Technology* 8:662).

A variety of host-expression vector systems may be utilized to express the antibody coding sequences of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified. but also produce cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, exhibit the antibody product of the invention in situ. These systems include, but are not limited to, microorganisms such as bacteria (*e.g.*, *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (*e.g.*, *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing the antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing antibody coding sequences; or mammalian cell systems (*e.g.*, COS, CHO, BHK, 293, and 3T3 cells) harboring recombinant expression constructs containing

promoters derived from the genome of mammalian cells (*e.g.*, the metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., 1983, *EMBO J.* 2:1791), in which the antibody coding sequence may be ligated individually into the vector in frame with the *lac Z* coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, *Nucleic Acids Res.* 13:3101-3109; Van Heeke & Schuster, 1989, *J. Biol. Chem.* 264:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to a matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in Spodoptera frugiperda cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody in infected hosts (*e.g.*, see Logan & Shenk, 1984, *Proc. Natl. Acad. Sci. USA* 81:3655-3659). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and

initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., 1987, *Methods in Enzymol.* 153:516-544).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, WI38.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., 1977, *Cell* 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1962, *Proc. Natl. Acad. Sci. USA* 48:2026), and adenine phosphoribosyltransferase (Lowy et al., 1980, *Cell* 22:817) genes can be employed in tk^-, $hgprt^-$ or $aprt^-$ cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., 1980, *Natl. Acad. Sci. USA* 77:3567; O'Hare et al., 1981, *Proc. Natl. Acad. Sci. USA* 78:1527); gpt, which confers resistance to mycophenolic acid

(Mulligan & Berg, 1981, *Proc. Natl. Acad. Sci. USA* 78:2072); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin et al., 1981, *J. Mol. Biol.* 150:1); and hygro, which confers resistance to hygromycin (Santerre et al., 1984, *Gene* 30:147).

The expression levels of the synthetic modified antibody can be increased by vector amplification (for a review, see Bebbington and Hentschel, *The Use of Vectors Based on Gene Amplification for the Expression of Cloned Genes in Mammalian Cells in DNA Cloning*, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing immunoglobulin is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the immunoglobulin gene, production of the immunoglobulin will also increase (Crouse et al., 1983, *Mol. Cell. Biol.* 3:257).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, 1986, *Nature* 322:562; Kohler, 1980, *Proc. Natl. Acad. Sci. USA* 77:2197). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

The invention provides a recombinant cell that contain a vector which encodes a synthetic antibody that has a CDR that contain the amino acid sequence of an active binding site from a member of a binding pair.

5.3. THERAPEUTIC USE OF SYNTHETIC MODIFIED ANTIBODIES

The invention also provides methods for treating or preventing diseases and disorders associated with the expression of a particular molecule by administration of a therapeutic of the invention (termed herein "Therapeutic"). Such Therapeutics include the modified immunoglobulins of the invention, and functionally active fragments thereof, (*e.g.*, as described in Section 5.1, *supra*), and nucleic acids encoding the modified immunoglobulins of the invention, and functionally active fragments thereof (*e.g.*, as described in Section 5.2, *supra.*).

Generally, administration of products of a species origin or species reactivity that is the same species as that of the subject is preferred. Thus, in preferred embodiments, the therapeutic methods of the invention use a modified antibody that is derived from a human

antibody; in other embodiments, the methods of the invention use a modified antibody that is derived from a chimeric or humanized antibody.

Specifically, pharmaceutical compositions containing the modified antibodies (or functionally active fragment thereof) of the invention that immunospecifically bind a particular molecule can be used in the treatment or prevention of diseases or disorders associated with the expression of the particular molecule, *e.g.*, an antigen. In particular, in embodiments discussed in more detail in the subsections that follow, modified antibodies that immunospecifically bind a tumor or cancer antigen or an antigen of an infectious disease agent or a cellular receptor for an infectious disease agent can be used to treat or prevent tumors, cancers or infectious diseases associated with the expression of the particular antigen. Modified immunoglobulins that immunospecifically bind a ligand or receptor can be used to treat or prevent a disease associated with a defect in decrease in or increase the amount of the particular ligand receptor. In certain embodiments, the modified immunoglobulins are used to treat or prevent autoimmune disease, including but not limited to rheumatoid arthritis, lupus, ulcerative colito, or psoriasis. The modified immunoglobulins may also be used to treat allergies.

The subjects to which the present invention is applicable may be any mammalian or vertebrate species, which include, but are not limited to, cows, horses, sheep, pigs, fowl (*e.g.*, chickens), goats, cats, dogs, hamsters, mice, rats, monkeys, rabbits, chimpanzees, and humans. In a preferred embodiment, the subject is a human.

5.3.1. TREATMENT AND PREVENTION OF CANCERS

The invention provides methods of treating or preventing cancers characterized by the presence of a particular cancer antigens which are a member of a binding pair. The method includes administering to a subject in need of such treatment or prevention a Therapeutic of the invention, *e.g.*, a synthetic modified antibody (or functionally active fragment thereof) that immunospecifically binds to the particular cancer antigen, which antibody comprises a variable domain with a CDR containing the amino acid sequence of a binding site for the cancer antigen.

Cancers, including, but not limited to, neoplasms, tumors, metastases, or any disease or disorder characterized by uncontrolled cell growth, can be treated or prevented by administration of the synthetic modified antibody of the invention, which modified antibody immunospecifically binds one or more antigens associated with the cancer cells of the cancer to be treated or prevented. Whether a particular Therapeutic is effective to treat or prevent a

certain type of cancer can be determined by any method known in the art, for example but not limited to, these methods described in Section 5.6, *infra*.

For example, but not by way of limitation, cancers and tumors associated with the following cancer and tumor antigens may be treated or prevented by administration of a synthetic antibody of the invention containing in its CDR the sequence that recognizes these cancer antigens: KS 1/4 pan-carcinoma antigen (Perez and Walker, 1990, *J. Immunol.* 142:3662-3667; Bumal, 1988, *Hybridoma* 7(4):407-415), ovarian carcinoma antigen (CA125) (Yu et al., 1991, *Cancer Res.* 51(2):468-475), prostatic acid phosphate (Tailor et al., 1990, *Nucl. Acids Res.* 18(16):4928), prostate specific antigen (Henttu and Vihko, 1989, *Biochem. Biophys. Res. Comm.* 160(2):903-910; Israeli et al., 1993, *Cancer Res.* 53:227-230), melanoma-associated antigen p97 (Estin et al., 1989, *J. Natl. Cancer Instit.* 81(6):445-446), melanoma antigen gp75 (Vijayasardahl et al., 1990, *J. Exp. Med.* 171(4):1375-1380), high molecular weight melanoma antigen (HMW-MAA) (Natali et al., 1987, *Cancer* 59:55-63; Mittelman et al., 1990, *J. Clin. Invest.* 86:2136-2144), prostate specific membrane antigen, carcinoembryonic antigen (CEA) (Foon et al., 1994, *Proc. Am. Soc. Clin. Oncol.* 13:294), polymorphic epithelial mucin antigen, human milk fat globule antigen, colorectal tumor-associated antigens such as: CEA, TAG-72 (Yokata et al., 1992, *Cancer Res.* 52:3402-3408), CO17-1A (Ragnhammar et al., 1993, *Int. J. Cancer* 53:751-758); GICA 19-9 (Herlyn et al., 1982, *J. Clin. Immunol.* 2:135), CTA-1 and LEA, Burkitt's lymphoma antigen-38.13, CD19 (Ghetie et al., 1994, *Blood* 83:1329-1336), human B-lymphoma antigen-CD20 (Reff et al., 1994, *Blood* 83:435-445), CD33 (Sgouros et al., 1993, *J. Nucl. Med.* 34:422-430), melanoma specific antigens such as ganglioside GD2 (Saleh et al., 1993, J.Immunol., 151, 3390-3398), ganglioside GD3 (Shitara et al., 1993, *Cancer Immunol. Immunother* 36:373-380), ganglioside GM2 (Livingston et al., 1994, *J. Clin. Oncol.* 12:1036-1044), ganglioside GM3 (Hoon et al., 1993, *Cancer Res.* 53:5244-5250), tumor-specific transplantation type of cell-surface antigen (TSTA) such as virally-induced tumor antigens including T-antigen DNA tumor viruses and Envelope antigens of RNA tumor viruses, oncofetal antigen-alpha-fetoprotein such as CEA of colon, bladder tumor oncofetal antigen (Hellstrom et al., 1985, *Cancer. Res.* 45:2210-2188), differentiation antigen such as human lung carcinoma antigen L6, L20 (Hellstrom et al., 1986, *Cancer Res.* 46:3917-3923), antigens of fibrosarcoma, human leukemia T cell antigen-Gp37 (Bhattacharya-Chatterjee et al., 1988, *J. of Immunospecifically.* 141:1398-1403), neoglycoprotein, sphingolipids, breast cancer antigen such as EGFR (Epidermal growth factor receptor), HER2 antigen ($p185^{HER2}$), polymorphic epithelial mucin (PEM) (Hilkens et al., 1992, *Trends in Bio. Chem. Sci.* 17:359), malignant human lymphocyte antigen-APO-1

(Bernhard et al., 1989, *Science* 245:301-304), differentiation antigen (Feizi, 1985, *Nature* 314:53-57) such as I antigen found in fetal erythrocytes, primary endoderm, I antigen found in adult erythrocytes, preimplantation embryos, I(Ma) found in gastric adenocarcinomas, M18, M39 found in breast epithelium, SSEA-1 found in myeloid cells, VEP8, VEP9, Myl, VIM-D5, $D_1$56-22 found in colorectal cancer, TRA-1-85 (blood group H), C14 found in colonic adenocarcinoma, F3 found in lung adenocarcinoma, AH6 found in gastric cancer, Y hapten, Le^y found in embryonal carcinoma cells, TL5 (blood group A), EGF receptor found in A431 cells, E_1 series (blood group B) found in pancreatic cancer, FC10.2 found in embryonal carcinoma cells, gastric adenocarcinoma antigen, CO-514 (blood group Le^a) found in Adenocarcinoma, NS-10 found in adenocarcinomas, CO-43 (blood group Le^b), G49 found in EGF receptor of A431 cells, MH2 (blood group ALe^b/Le^y) found in colonic adenocarcinoma, 19.9 found in colon cancer, gastric cancer mucins, T_5A_7 found in myeloid cells, R_{24} found in melanoma, 4.2, G_{D3}, D1.1, OFA-1, G_{M2}, OFA-2, G_{D2}, and M1:22:25:8 found in embryonal carcinoma cells, and SSEA-3 and SSEA-4 found in 4 to 8-cell stage embryos. In one embodiment, the antigen is a Tcell receptor derived peptide from a Cutaneous Tcell Lymphoma (see, Edelson, 1998, The Cancer Journal 4:62).

In other embodiments of the invention, the subject being treated with the modified antibody may, optionally, be treated with other cancer treatments such as surgery, radiation therapy or chemotherapy. In particular, the Therapeutic of the invention used to treat or prevent cancer may be administered in conjunction with one or a combination of chemotherapeutic agents including, but not limited to, methotrexate, taxol, mercaptopurine, thioguanine, hydroxyurea, cytarabine, cyclophosphamide, ifosfamide, nitrosoureas, cisplatin, carboplatin, mitomycin, dacarbazine, procarbizine, etoposides, campathecins, bleomycin, doxorubicin, idarubicin, daunorubicin, dactinomycin, plicamycin, mitoxantrone, asparaginase, vinblastine, vincristine, vinorelbine, paclitaxel, docetaxel, etc. In a preferred embodiment, the synthetic modified antibody is conjugated to a chemotherapeutic agent or other type of toxin, *e.g.*, a ricin toxin, or a radionuclide, or any other agent effective to kill cancer or tumor cells or to arrest cancer cell growth. In another preferred embodiment, the modified immunoglobulin has one CDR containing a binding site for a cancer antigen and another CDR containing a binding site for molecule on the surface of an immune cell, such as but not limited to a T cell, a B cell, NK cell, K cell, TIL cell or neutrophil.

In certain embodiments of the invention where the CDR of the synthetic modified antibody includes an amino acid sequence that immunospecifically binds a human colon carcinoma-associated protein antigen, it is preferred that the antibody has the following characteristics: (i) the antibody recognizes epitopes of a protein component of the antigen,

but does not recognize the epitopes of the carbohydrate component(s) of the antigen; (ii) the antigen is not detectable on normal human tissue; and (iii) the antigen is not detectable on human carcinoma cells other than colon carcinoma cells. In other embodiments, the CDR of the synthetic modified antibody includes an amino acid sequence that immunospecifically binds an antigen which is not detectable on human carcinoma cells other than breast carcinoma cells. In yet other embodiments, the CDR of the synthetic modified antibody includes an amino acid sequence that immunospecifically binds an antigen is not detectable on human carcinoma cells other than ovarian carcinoma cells.

5.3.1.1. MALIGNANCIES

Malignancies and related disorders that can be treated or prevented by administration of a Therapeutic of the invention include but are not limited to those listed in Table 2 (for a review of such disorders, see Fishman et al., 1985, *Medicine*, 2d Ed., J.B. Lippincott Co., Philadelphia):

TABLE 3

MALIGNANCIES AND RELATED DISORDERS

- Leukemia
  - acute leukemia
    - acute lymphocytic leukemia
    - acute myelocytic leukemia
      - myeloblastic
      - promyelocytic
      - myelomonocytic
      - monocytic
      - erythroleukemia
  - chronic leukemia
    - chronic myelocytic (granulocytic) leukemia
    - chronic lymphocytic leukemia
- Polycythemia vera
- Lymphoma
  - Hodgkin's disease
  - non-Hodgkin's disease
- Multiple myeloma
- Waldenström's macroglobulinemia
- Heavy chain disease
- Solid tumors
  - sarcomas and carcinomas
    - fibrosarcoma
    - myxosarcoma
    - liposarcoma

chondrosarcoma
osteogenic sarcoma
chordoma
angiosarcoma
endotheliosarcoma
lymphangiosarcoma
lymphangioendotheliosarcoma
synovioma
mesothelioma
Ewing's tumor
leiomyosarcoma
rhabdomyosarcoma
colon carcinoma
pancreatic cancer
breast cancer
ovarian cancer
prostate cancer
squamous cell carcinoma
basal cell carcinoma
adenocarcinoma
sweat gland carcinoma
sebaceous gland carcinoma
papillary carcinoma
papillary adenocarcinomas
cystadenocarcinoma
medullary carcinoma
bronchogenic carcinoma
renal cell carcinoma
hepatoma
bile duct carcinoma
choriocarcinoma
seminoma
embryonal carcinoma
Wilms' tumor
cervical cancer
uterine cancer
testicular tumor
lung carcinoma
small cell lung carcinoma
bladder carcinoma
epithelial carcinoma
glioma
astrocytoma
medulloblastoma
craniopharyngioma
ependymoma
pinealoma
hemangioblastoma
acoustic neuroma
oligodendroglioma
meningioma
melanoma

neuroblastoma
retinoblastoma

In specific embodiments, malignancy or dysproliferative changes (such as metaplasias and dysplasias), or hyperproliferative disorders, are treated or prevented in the ovary, bladder, breast, colon, lung, skin, pancreas, prostate, uterus, gastrointestinal tract, B lymphocytes or T lymphocytes. In other specific embodiments, sarcoma. melanoma, or leukemia is treated or prevented.

5.3.1.2. PREMALIGNANT CONDITIONS

The Therapeutics of the invention can also be administered to treat premalignant conditions and to prevent progression to a neoplastic or malignant state, including, but not limited to, those disorders listed in Table 3. Such prophylactic or therapeutic use is indicated in conditions known or suspected of preceding progression to neoplasia or cancer, in particular, where non-neoplastic cell growth consisting of hyperplasia, metaplasia, or most particularly, dysplasia has occurred (for review of such abnormal growth conditions, see Robbins and Angell, 1976, *Basic Pathology*, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 68-79.) Hyperplasia is a form of controlled cell proliferation involving an increase in cell number in a tissue or organ, without significant alteration in structure or function. As but one example, endometrial hyperplasia often precedes endometrial cancer. Metaplasia is a form of controlled cell growth in which one type of adult or fully differentiated cell substitutes for another type of adult cell. Metaplasia can occur in epithelial or connective tissue cells. Atypical metaplasia involves a somewhat disorderly metaplastic epithelium. Dysplasia is frequently a forerunner of cancer, and is found mainly in the epithelia; it is the most disorderly form of non-neoplastic cell growth, involving a loss in individual cell uniformity and in the architectural orientation of cells. Dysplastic cells often have abnormally large, deeply stained nuclei, and exhibit pleomorphism. Dysplasia characteristically occurs where there exists chronic irritation or inflammation, and is often found in the cervix, respiratory passages, oral cavity, and gall bladder.

Alternatively or in addition to the presence of abnormal cell growth characterized as hyperplasia, metaplasia, or dysplasia, the presence of one or more characteristics of a transformed phenotype, or of a malignant phenotype, displayed *in vivo* or displayed *in vitro* by a cell sample from a patient, can indicate the desirability of prophylactic/therapeutic administration of a Therapeutic. As mentioned *supra*, such characteristics of a transformed

phenotype include morphology changes, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, protease release, increased sugar transport, decreased serum requirement, expression of fetal antigens, disappearance of the 250,000 dalton cell surface protein, etc. (see also *id.*, at pp. 84-90 for characteristics associated with a transformed or malignant phenotype).

In a specific embodiment, leukoplakia, a benign-appearing hyperplastic or dysplastic lesion of the epithelium, or Bowen's disease, a carcinoma *in situ*, are pre-neoplastic lesions indicative of the desirability of prophylactic intervention.

In another embodiment, fibrocystic disease (cystic hyperplasia, mammary dysplasia, particularly adenosis (benign epithelial hyperplasia)) is indicative of the desirability of prophylactic intervention.

In other embodiments, a patient which exhibits one or more of the following predisposing factors for malignancy is treated by administration of an effective amount of the Therapeutic of the invention: a chromosomal translocation associated with a malignancy (*e.g.*, the Philadelphia chromosome for chronic myelogenous leukemia, t(14;18) for follicular lymphoma, etc.), familial polyposis or Gardner's syndrome (possible forerunners of colon cancer), benign monoclonal gammopathy (a possible forerunner of multiple myeloma), and a first degree kinship with persons having a cancer or precancerous disease showing a Mendelian (genetic) inheritance pattern (*e.g.*, familial polyposis of the colon, Gardner's syndrome, hereditary exostosis, polyendocrine adenomatosis, medullary thyroid carcinoma with amyloid production and pheochromocytoma, Peutz-Jeghers syndrome, neurofibromatosis of Von Recklinghausen, retinoblastoma, carotid body tumor, cutaneous melanocarcinoma, intraocular melanocarcinoma, xeroderma pigmentosum, ataxia telangiectasia, Chediak-Higashi syndrome, albinism, Fanconi's aplastic anemia, and Bloom's syndrome; *see* Robbins and Angell, 1976, *Basic Pathology*, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 112-113) etc.)

In another specific embodiment, Therapeutics of the invention is administered to a human patient to prevent progression to ovary, breast, colon, lung, pancreatic, bladder, skin, prostate, colon, gastrointestinal, B lymphocyte, T lymphocyte or uterine cancer, or melanoma or sarcoma.

5.3.2. TREATMENT OF INFECTIOUS DISEASE

The invention also provides methods of treating or preventing an infectious disease by administration of a Therapeutic of the invention, in particular, a synthetic modified immunoglobulin (or the functionally active fragment thereof) that immunospecifically binds

an antigen of the agent causing the infectious disease or a cellular receptor for the infectious disease agent, or an enzyme expressed by the infectious diseases agent. As discussed in detail below, the infectious agents include, but are not limited to, viruses, bacteria, fungi, protozoa, and parasites.

In specific embodiments, infectious diseases are treated or prevented by administration of a modified antibody of the immunoglobin (or functionally active fragment thereof) that immunospecifically recognizes one of the following antigens of an infectious disease agent: influenza virus hemagglutinin (Genbank accession no. JO2132; Air, 1981, *Proc. Natl. Acad. Sci. USA* 78:7639-7643; Newton et al., 1983, *Virology* 128:495-501), human respiratory syncytial virus G glycoprotein (Genbank accession no. Z33429; Garcia et al., 1994, *J. Virol.*; Collins et al., 1984, *Proc. Natl. Acad. Sci. USA* 81:7683), core protein, matrix protein or other protein of Dengue virus (Genbank accession no. M19197; Hahn et al., 1988, *Virology* 162:167-180), measles virus hemagglutinin (Genbank accession no. M81899; Rota et al., 1992, *Virology* 188:135-142), herpes simplex virus type 2 glycoprotein gB (Genbank accession no. M14923; Bzik et al., 1986, *Virology* 155:322-333), poliovirus I VP1 (Emini et al., 1983, *Nature* 304:699), envelope glycoproteins of HIV I (Putney et al., 1986, *Science* 234:1392-1395), hepatitis B surface antigen (Itoh et al., 1986, *Nature* 308:19; Neurath et al., 1986, *Vaccine* 4:34), diptheria toxin (Audibert et al., 1981, *Nature* 289:543), streptococcus 24M epitope (Beachey, 1985, *Adv. Exp. Med. Biol.* 185:193), gonococcal pilin (Rothbard and Schoolnik, 1985, *Adv. Exp. Med. Biol.* 185:247), pseudorabies virus g50 (gpD), pseudorabies virus II (gpB), pseudorabies virus gIII (gpC), pseudorabies virus glycoprotein H, pseudorabies virus glycoprotein E, transmissible gastroenteritis glycoprotein 195, transmissible gastroenteritis matrix protein, swine rotavirus glycoprotein 38, swine parvovirus capsid protein, *Serpulina hydodysenteriae* protective antigen, bovine viral diarrhea glycoprotein 55, Newcastle disease virus hemagglutinin-neuraminidase, swine flu hemagglutinin, swine flu neuraminidase, foot and mouth disease virus, hog colera virus, swine influenza virus, African swine fever virus, *Mycoplasma hyopneumoniae*, infectious bovine rhinotracheitis virus (*e.g.*, infectious bovine rhinotracheitis virus glycoprotein E or glycoprotein G), or infectious laryngotracheitis virus (*e.g.*, infectious laryngotracheitis virus glycoprotein G or glycoprotein I), a glycoprotein of La Crosse virus (Gonzales-Scarano et al., 1982, *Virology* 120:42), neonatal calf diarrhea virus (Matsuno and Inouye, 1983, *Infection and Immunity* 39:155), Venezuelan equine encephalomyelitis virus (Mathews and Roehrig, 1982, *J. Immunol.* 129:2763), punta toro virus (Dalrymple et al., 1981, *in* Replication of Negative Strand Viruses, Bishop and Compans (eds.), Elsevier, NY, p. 167), murine leukemia virus (Steeves et al., 1974, *J. Virol.* 14:187), mouse mammary tumor virus

(Massey and Schochetman, 1981, *Virology* 115:20), hepatitis B virus core protein and/or hepatitis B virus surface antigen or a fragment or derivative thereof (see, *e.g.*, U.K. Patent Publication No. GB 2034323A published June 4, 1980; Ganem and Varmus, 1987, *Ann. Rev. Biochem.* 56:651-693; Tiollais et al., 1985, *Nature* 317:489-495), antigen of equine influenza virus or equine herpesvirus (*e.g.*, equine influenza virus type A/Alaska 91 neuraminidase, equine influenza virus type A/Miami 63 neuraminidase, equine influenza virus type A/Kentucky 81 neuraminidase equine herpesvirus type 1 glycoprotein B, and equine herpesvirus type 1 glycoprotein D, antigen of bovine respiratory syncytial virus or bovine parainfluenza virus (*e.g.*, bovine respiratory syncytial virus attachment protein (BRSV G), bovine respiratory syncytial virus fusion protein (BRSV F), bovine respiratory syncytial virus nucleocapsid protein (BRSV N), bovine parainfluenza virus type 3 fusion protein, and the bovine parainfluenza virus type 3 hemagglutinin neuraminidase), bovine viral diarrhea virus glycoprotein 48 or glycoprotein 53.

Cellular receptors that can be targeted for treatment of an infectious disease are listed in Table 4, along with the pathogen which binds to the cellular receptor.

TABLE 4

Pathogen	**Cellular Receptor**
B-lymphotropic papovavirus (LPV)	LPV receptor on B-cells
Bordatella pertussis	Adenylate cyclase
Borna Disease virus (BDV)	BDV surface glycoproteins
Bovine coronavirus	N-acetyl-9-O-acetylneuraminic acid receptor
Choriomeningitis virus	CD4+
Dengue virus	Highly sulphated type Heparin sulphate p65
E. coli	Gal alpha 1-4Gal-containing isoreceptors
Ebola	CD16b
Echovirus 1	Integrin VLA-2 receptor
Echovirus-11 (EV)	EV receptor
Endotoxin (LPS)	CD14
Enteric bacteria	Glycoconjugate receptors
Enteric Orphan virus	alpha/beta T-cell receptor

Pathogen	Cellular Receptor
Enteroviruses	Decay-accelerating factor receptor
Feline leukemia virus	Extracellular envelope glycoprotein (Env-SU) receptor
Foot and mouth disease virus	Immunoglobulin Fc receptorPoxvirusM-T7
Gibbon ape leukemia virus (GALV)	GALV receptor
Gram-negative bacteria	CD14 receptor
Heliobacter pylori	Lewis(b) blood group antigen receptor
Hepatitis B virus (HBV)	T-cell receptor
Herpes Simplex Virus	Heparin sulphate glycoaminoglycan receptor Fibroblast growth factor receptor
HIV-1	CC-Chemokine receptor CCR5 CD11a CD2 G-protein coupled receptor CD4
Human cytomegalovirus	Heparin sulphate proteoglycan Annexin II CD13 (aminopeptidase N)
Human coronovirus	Human aminopeptidase N receptor
Influenza A, B & C	Hemagglutinin receptor
Legionella	CR3 receptor Protein kinase receptor Galactose N-acetylgalactosamine (Gal/GalNAc)-inhibitable lectin receptor Chemokine receptor
Leishmania mexicana	Annexin I
Listeria monocytogenes	ActA protein
Measles virus	CD46 receptor
Meningococcus	Meningococcal virulence associated Opa receptors

Pathogen	Cellular Receptor
Morbilliviruses	CD46 receptor
Mouse hepatitis virus	Carcinoembryonic antigen family receptors Carcinoembryonic antigen family Bg1a receptor
Murine leukemia virus	Envelope glycoproteins
Murine gamma herpes virus	gamma interferon receptor
Murine retrovirus	Glycoprotein gp70 Rmc-1 receptor
Murine coronavirus mouse hepatitis virus	Carcinoembryonic antigen family receptors
Mycobacterium avium-M	Human Integrin receptor alpha v beta 3
Neisseria gonorrhoeae	Heparin sulphate proteoglycan receptor CD66 receptor Integrin receptor Membrane cofactor protein CD46 GM1 GM2 GM3 CD3 Ceramide
Newcastle disease virus	Hemagglutinin-neuraminidase protein Fusion protein
Parvovirus B19	Erythrocyte P antigen receptor
Plasmodium falciparum	CD36 receptor Glycophorin A receptor
Pox Virus	Interferon gamma receptor
Pseudomonas	KDEL receptor
Rotavirus	Mucosal homing alpha4beta7 receptor
Samonella typhiurium	Epidermal growth factor receptor

Pathogen	Cellular Receptor
Shigella	alpha5beta1 integrin protein
Streptococci	Nonglycosylated J774 receptor
T-helper cells type 1	Chemokine receptors including: 6.CXCR1-4 7.CCR1-5 8.CXCR3 9.CCR5
T-cell lymphotropic virus 1	gp46 surface glycoprotein
Vaccinia virus	TNFRp55 receptor TNFRp75 receptor Soluble Interleukin-1 beta receptor

Viral diseases that can be treated or prevented by the methods of the present invention include, but are not limited to, those caused by hepatitis type A, hepatitis type B, hepatitis type C, influenza, varicella, adenovirus, herpes simplex type I (HSV-I), herpes simplex type II (HSV-II), rinderpest, rhinovirus, echovirus, rotavirus, respiratory syncytial virus, papilloma virus, papova virus, cytomegalovirus, echinovirus, arbovirus, hantavirus, coxsachie virus, mumps virus, measles virus, rubella virus, polio virus, human immunodeficiency virus type I (HIV-I), and human immunodeficiency virus type II (HIV-II), any picornaviridae, enteroviruses, caliciviridae, any of the Norwalk group of viruses, togaviruses, such as Dengue virus, alphaviruses, flaviviruses, coronaviruses, rabies virus, Marburg viruses, ebola viruses, parainfluenza virus, orthomyxoviruses, bunyaviruses, arenaviruses, reoviruses, rotaviruses, orbiviruses, human T cell leukemia virus type I, human T cell leukemia virus type II, simian immunodeficiency virus, lentiviruses, polyomaviruses, parvoviruses, Epstein-Barr virus, human herpesvirus-6, cercopithecine herpes virus 1 (B virus), poxviruses, and encephalitis.

Bacterial diseases that can be treated or prevented by the methods of the present invention are caused by bacteria including, but not limited to, gram negative and gram positive bacteria, mycobacteria rickettsia, mycoplasma, Shigella spp., Neisseria spp. (*e.g.*, *Neisseria mennigitidis* and *Neisseria gonorrhoeae*), legionella, *Vibrio cholerae*, Streptococci, such as *Streptococcus pneumoniae*, *corynebacteria diphtheriae*, *clostridium*

tetani, bordetella pertussis, Haemophilus spp. (*e.g.*, *influenzae*), Chlamydia spp., Enterotoxigenic *Escherichia coli*, etc. and bacterial diseases Syphillis. Lyme's desease, etc.

Protozoal diseases that can be treated or prevented by the methods of the present invention are caused by protozoa including, but not limited to, plasmodia, eimeria, leishmania, kokzidioa, and trypanosoma, fungi, such as Candida, etc.

In specific embodiments of the invention, the Therapeutic is administered in conjunction with an appropriate antibiotic, antifungal, anti-viral or any other drug useful in treating or preventing the infectious disease. In a preferred embodiment, the synthetic modified antibody is conjugated to a compound effective against the infectious disease agent to which the synthetic modified antibody is directed, for example, an antibiotic, antifungal or anti-viral agent. In another preferred embodiment, the modified immunoglobulin has one CDR containing a binding site for an antigen of an infectious disease agent and another CDR containing a binding site for a molecule on the surface of an immune cell, such as but not limited to a T cell, a B cell, NK cell, K cell, TIL cell or neutrophil.

5.3.3. GENE THERAPY

In a specific embodiment, nucleic acids comprising a sequence encoding a synthetic modified antibody of the invention are administered to treat or prevent a disease or disorder associated with the expression of a molecule to which the synthetic modified antibody immunospecifically binds.

In this embodiment of the invention, the therapeutic nucleic acid encodes a sequence that produces intracellularly (without a leader sequence) or intercellularly (with a leader sequence) a modified immunoglobulin of the invention.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel et al., 1993, *Clinical Pharmacy* 12:488-505; Wu and Wu, 1991, *Biotherapy* 3:87-95; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596; Mulligan, 1993, *Science* 260:926-932; and Morgan and Anderson, 1993, *Ann. Rev. Biochem.* 62:191-217; May, 1993, *TIBTECH* 11(5):155-215). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), 1993, Current Protocols in Molecular Biology, John Wiley & Sons, NY; Kriegler, 1990, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY; and in Chapters 12 and 13, Dracopoli et al. (eds), 1994, Current Protocols in Human Genetics, John Wiley & Sons, NY.

In one aspect, the therapeutic nucleic acid comprises an expression vector that expresses the modified immunoglobulin (or fragment thereof) in a suitable host. In particular, such a nucleic acid has a promoter operably linked to the coding sequence for the modified synthetic antibody, said promoter being inducible or constituitive, and, optionally, tissue-specific. In another embodiment, a nucleic acid molecule is used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the modified antibody (Koller and Smithies, 1989, *Proc. Nat'l. Acad. Sci. USA* 86:8932-8935; Zijlstra et al., 1989, *Nature* 342:435-438).

Delivery of the nucleic acid into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vector or a delivery complex, or indirect, in which case, cells are first transformed with the nucleic acid *in vitro*, then transplanted into the patient. These two approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy.

In a specific embodiment, the nucleic acid is directly administered *in vivo*, where it is expressed to produce the antibodies. This can be accomplished by any of numerous methods known in the art, *e.g.*, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by infection using a defective or attenuated retroviral or other viral vector (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont); or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in biopolymers (*e.g.*, poly-ß-1->4-N-acetylglucosamine polysaccharide; see U.S. Patent No. 5,635,493), encapsulation in liposomes, microparticles, or microcapsules, or by administering it in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see *e.g.*, Wu and Wu, 1987, *J. Biol. Chem.* 262:4429-4432), etc. In another embodiment, a nucleic acid-ligand complex can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, *e.g.*, PCT Publications WO 92/06180 dated April 16, 1992 (Wu et al.); WO 92/22635 dated December 23, 1992 (Wilson et al.); WO92/20316 dated November 26, 1992 (Findeis et al.); WO93/14188 dated July 22, 1993 (Young). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host

cell DNA for expression, by homologous recombination (Koller and Smithies, 1989, *Proc. Natl. Acad. Sci. USA* 86:8932-8935; Zijlstra et al., 1989, *Nature* 342:435-438).

Alternatively, single chain antibodies can also be administered, for example, by expressing nucleotide sequences encoding single-chain antibodies within the target cell population by utilizing, for example, techniques such as those described in Marasco et al. (Marasco et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:7889-7893). Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, 1993, *Current Opinion in Genetics and Development* 3:499-503 present a review of adenovirus-based gene therapy. Bout et al., 1994, *Human Gene Therapy* 5:3-10 demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., 1991, *Science* 252:431-434; Rosenfeld et al., 1992, *Cell* 68:143-155; and Mastrangeli et al., 1993, *J. Clin. Invest.* 91:225-234. Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., 1993, *Proc. Soc. Exp. Biol. Med.* 204:289-300).

The form and amount of therapeutic nucleic acid envisioned for use depends on the type of disease and the severity of the desired effect, patient state, etc., and can be determined by one skilled in the art.

5.3.4. VACCINE IMMUNIZATION

The modified antibody of the present invention may be used as a vaccine in a subject in which immunity for the binding site for the particular molecule or antigen is desired. The vaccines and methods of the present invention may be used either to prevent a disease or disorder, or to treat a particular disease or disorder, where an anti-idiotype response against a particular synthetic antibody is therapeutically or prophylactically useful.

The methods and compositions of the present invention may be used to elicit a humoral and/or a cell-mediated response against the synthetic antibody of the vaccine in a subject. In one specific embodiment, the methods and compositions elicit a humoral response against the administered synthetic antibody in a subject. In another specific embodiment, the methods and compositions elicit a cell-mediated response against the administered synthetic antibody in a subject. In a preferred embodiment, the methods and compositions elicit both a humoral and a cell-mediated response.

5.4. PHARMACEUTICAL PREPARATIONS AND METHODS OF ADMINISTRATION

5.4.1. FORMULATIONS AND ADMINISTRATION

Therapeutic compositions containing a modified immunoglobulin for use in accordance with the present invention can be formulated in any conventional manner using one or more physiologically acceptable carriers or excipients.

Thus, the modified immunoglobulins (or functionally active fragments thereof or nucleic acids encoding the antibodies or fragments) and their physiologically acceptable salts and solvents can be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, buccal, parenteral or rectal administration.

For oral administration, the Therapeutics can take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulphate). The tablets can be coated by methods well known in the art. Liquid preparations for oral administration can take the form of, for example, solutions, syrups or suspensions, or they can be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations can be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations can also contain buffer salts, flavoring, coloring and sweetening agents as appropriate.

Preparations for oral administration can be suitably formulated to give controlled release of the active compound.

For buccal administration the Therapeutics can take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the Therapeutics according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit can be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, *e.g.*, gelatin for use in an inhaler or

insufflator can be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The Therapeutics can be formulated for parenteral administration (*i.e.*, intravenous or intramuscular) by injection, via, for example, bolus injection or continuous infusion. Formulations for injection can be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions can take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient can be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The Therapeutics can also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the Therapeutics can also be formulated as a depot preparation. Such long acting formulations can be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds can be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The modified immunoglobulins of the invention may be administered as separate compositions or as a single composition with more than one antibodies linked by conventional chemical or by molecular biological methods. Additionally, the diagnostic and therapeutic value of the antibodies of the invention may be augmented by their use in combination with radionuclides or with toxins such as ricin or with chemotherapeutic agents such as methotrexate.

The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc.

Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is administered by injection, an ampoule of sterile diluent can be provided so that the ingredients may be mixed prior to administration.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the vaccine formulations of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. Composition comprising a compound of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labelled for treatment of an indicated condition.

Many methods may be used to introduce the vaccine formulations of the invention; these include but are not limited to oral, intracerebral, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal routes, and via scarification (scratching through the top layers of skin, *e.g.*, using a bifurcated needle) or any other standard routes of immunization.

The precise dose of the modified immunoglobulin molecule to be employed in the formulation will also depend on the route of administration, and the nature of the patient, and should be decided according to the judgment of the practitioner and each patient's circumstances according to standard clinical techniques. An effective immunizing amount is that amount sufficient to produce an immune response to the synthetic antibody in the host to which the vaccine preparation is administered. Effective doses may also be extrapolated from dose-response curves derived from animal model test systems.

5.4.2. EFFECTIVE DOSE

The compounds and nucleic acid sequences described herein can be administered to a patient at therapeutically effective doses to treat certain diseases or disorders such as cancers or infectious diseases. A therapeutically effective dose refers to that amount of a compound sufficient to result in a healthful benefit in the treated subject.

Toxicity and therapeutic efficacy of compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is

the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects can be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

5.4.3. VACCINE FORMULATIONS AND ADMINISTRATION

The invention also provides vaccine formulations containing Therapeutics of the invention, which vaccine formulations are suitable for administration to elicit a protective immune (humoral and/or cell mediated) response against certain antigens , *e.g.*, for the treatment and prevention of diseases.

Suitable preparations of such vaccines include injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, suspension in, liquid prior to injection, may also be prepared. The preparation may also be emulsified, or the polypeptides encapsulated in liposomes. The active immunogenic ingredients are often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, buttered saline, dextrose, glycerol, ethanol, sterile isotonic aqueous buffer or the like and combinations thereof. In addition, if desired, the vaccine preparation may also include minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine.

Examples of adjuvants which may be effective, include, but are not limited to: aluminim hydroxide, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-

nor-muramyl-L-alanyl-D-isoglutamine, N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine.

The effectiveness of an adjuvant may be determined by measuring the induction of anti-idiotype antibodies directed against the injected immunoglobulin formulated with the particular adjuvant.

The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc.

Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is administered by injection, an ampoule of sterile diluent can be provided so that the ingredients may be mixed prior to administration.

In a specific embodiment, the lyophilized modified immunoglobulin of the invention is provided in a first container; a second container comprises diluent consisting of an aqueous solution of 50% glycerin, 0.25% phenol, and an antiseptic (*e.g.*, 0.005% brilliant green).

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the vaccine formulations of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. Composition comprising a compound of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

The subject to which the vaccine is administered is preferably a mammal, most preferably a human, but can also be a non-human animal including but not limited to cows, horses, sheep, pigs, fowl (*e.g.*, chickens), goats, cats, dogs, hamsters, mice and rats.

Many methods may be used to introduce the vaccine formulations of the invention; these include but are not limited to oral, intracerebral, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal routes, and via scarification (scratching through the top layers of skin, *e.g.*, using a bifurcated needle) or any other standard routes of immunization. In a specific embodiment, scarification is employed.

The precise dose of the modified immunoglobulin molecule to be employed in the formulation will also depend on the route of administration, and the nature of the patient, and should be decided according to the judgment of the practitioner and each patient's circumstances according to standard clinical techniques. An effective immunizing amount is that amount sufficient to produce an immune response to the modified immunoglobulin molecule in the host (*i.e.*, an anti-idiotype reaction) to which the vaccine preparation is administered. Effective doses may also be extrapolated from dose-response curves derived from animal model test systems.

5.5. DIAGNOSTIC METHODS

Modified immunoglobulins, particularly antibodies, (and functionally active fragments thereof) that bind a specific molecule that is a member of a binding pair may be used as diagnostics and prognostics, as described herein. In various embodiments, the present invention provides the measurement of a member of the binding pair, and the uses of such measurements in clinical applications. The modified immunoglobulins in the present invention may be used, for example, in the detection of an antigen in a biological sample whereby patients may be tested for aberrant levels of the molecule to which the modified immunoglobulin binds, and/or for the presence of abnormal forms of such molecules. By "aberrant levels" is meant increased or decreased relative to that present, or a standard level representing that present, in an analogous sample from a portion of the body or from a subject not having the disorder. The modified antibodies of this invention may also be included as a reagent in a kit for use in a diagnostic or prognostic technique.

In the specific embodiments of the invention, a modified antibody of the invention that immunospecifically binds to a cancer or tumor antigen or an antigen of an infectious disease agent may be used to diagnose, prognose or screen for a cancer or tumor or an infectious disease associated with the expression of the cancer or tumor antigen or the antigen of the infectious disease agent. In a preferred aspect, the invention provides a method of diagnosing or screening for the presence of or a predisposition for developing a cancer characterized by the increased presence of a cancer antigen, which is a first member

of a binding pair consisting of said first member and a second member, said method comprising measuring in a subject the level of immunospecific binding of a modified antibody to a sample derived from the subject, in which said modified antibody immunospecifically binds said cancer antigen and in which said modified antibody comprises a variable domain having at least one CDR containing portion of said second member, said portion containing a binding site for said cancer antigen and not being found naturally within said CDR, in which an increase in the level of said immunospecific binding, relative to the level of said immunospecific binding in an analogous sample from a subject not having the cancer or a predisposition for developing the cancer, indicates the presence of the cancer or a predisposition for developing the cancer.

In another preferred aspect, the invention provides a method of diagnosing or screening for the presence of an infectious disease agent, characterized by the presence of an antigen of said infectious disease agent, which antigen is a first member of a binding pair consisting of said first member and a second member, said method comprising measuring in a subject the level of immunospecific binding of a modified antibody to a sample derived from the subject, in which said modified antibody immunospecifically binds said antigen and in which said modified antibody comprises a variable domain having at least one CDR containing an at least four amino acid portion of said second member, said portion containing a binding site for said antigen and not being found naturally within said CDR, in which an increase in the level of said immunospecific binding, relative to the level of said immunospecific binding in an analogous sample from a subject not having the infectious disease agent, indicates the presence of said infectious disease agent.

In another preferred embodiment, the invention provides a method for detecting abnormal levels of a particular ligand or receptor in a sample derived from a subject by comparing the immunospecific binding of a modified antibody that binds the particular ligand or receptor to the sample with the immunospecific binding of the modified antibody to a sample having normal levels of the ligand or receptor.

The measurement of a molecule that is bound by a modified antibody can be valuable in detecting and/or staging diseases related to the molecule in a subject, in screening of such diseases in a population, in differential diagnosis of the physiological condition of a subject, and in monitoring the effect of a therapeutic treatment on a subject.

The following assays are designed to detect molecules to which the modified antibodies immunospecifically bind.

In specific embodiments, these diagnostic methods may be used to detect abnormalities in the level of gene expression, or abnormalities in the structure and/or temporal, tissue, cellular, or subcellular location of the particular molecule to be assayed.

The tissue or cell type to be analyzed will generally include those which are known, or suspected, to express the particular molecule. The protein isolation methods employed herein may, for example, be such as those described in Harlow and Lane (Harlow, E. and Lane, D., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). The isolated cells can be derived from cell culture or from a patient. The modified antibodies (or functionally active fragments thereof) useful in the present invention may, additionally, be employed histologically, as in immunofluorescence or immunoelectron microscopy, for *in situ* detection of the molecule. *In situ* detection may be accomplished by removing a histological specimen from a patient, such as paraffin embedded sections of affected tissues and applying thereto a labeled modified antibody of the present invention. The modified antibody (or functionally active fragment thereof) is preferably applied by overlaying the labeled modified antibody onto a biological sample. If the molecule to which the antibody binds is present in the cytoplasm, it may be desirable to introduce the modified antibody inside the cell, for example, by making the cell membrane permeable. Through the use of such a procedure, it is possible to determine not only the presence of the particular molecule, but also its distribution in the examined tissue. Using the present invention, those of ordinary skill will readily perceive that any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such *in situ* detection.

Immunoassays for the particular molecule will typically comprise incubating a sample, such as a biological fluid, a tissue extract, freshly harvested cells, or lysates of cultured cells, in the presence of a detectably labeled modified antibody and detecting the bound antibody by any of a number of techniques well-known in the art.

The biological sample may be brought in contact with and immobilized onto a solid phase support or carrier such as nitrocellulose, or other solid support which is capable of immobilizing cells, cell particles or soluble proteins. The support may then be washed with suitable buffers followed by treatment with the detectably labeled modified antibody. The solid phase support may then be washed with the buffer a second time to remove unbound antibody. The amount of bound label on solid support may then be detected by conventional means.

By "solid phase support or carrier" is intended any support capable of binding an antigen or an antibody. Well-known supports or carriers include glass, polystyrene,

polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or antibody. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

The binding activity of a given modified antibody may be determined according to well known methods. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

One of the ways in which a modified antibody can be detectably labeled is by linking the same to an enzyme and use in an enzyme immunoassay (EIA) (Voller, A., "The Enzyme Linked Immunosorbent Assay (ELISA)", 1978, *Diagnostic Horizons* 2:1-7, Microbiological Associates Quarterly Publication, Walkersville, MD); Voller et al., 1978, *J. Clin. Pathol.* 31:507-520; Butler, 1981, *Meth. Enzymol.* 73:482-523; Maggio, E. (ed.), 1980, Enzyme Immunoassay, CRC Press, Boca Raton, FL,; Ishikawa et al., (eds.), 1981, Enzyme Immunoassay, Kgaku Shoin, Tokyo)). The enzyme which is bound to the modified antibody will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorimetric or by visual means. Enzymes which can be used to detectably label the modified antibody include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate, dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase. The detection can be accomplished by colorimetric methods which employ a chromogenic substrate for the enzyme. Detection may also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

Detection may also be accomplished using any of a variety of other immunoassays. For example, by radioactively labeling the synthetic antibodies or fragments, it is possible to detect the protein that the antibody was designed for through the use of a radioimmunoassay

(RIA) (see, for example, Weintraub, 1986, Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society). The radioactive isotope can be detected by such means as the use of a gamma counter or a scintillation counter or by autoradiography.

It is also possible to label the modified antibody with a fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wave length, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent labeling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

The modified antibody can also be detectably labeled using fluorescence emitting metals such as ^{152}Eu, or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

The modified antibody also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antibody is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

Likewise, a bioluminescent compound may be used to label the synthetic modified antibody of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems, in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

5.6. DEMONSTRATION OF THERAPEUTIC UTILITY

The Therapeutics of the invention are preferably tested *in vitro*, and then *in vivo*, for the desired therapeutic or prophylactic activity, prior to use in humans. For example, *in vitro* assays that can be used to determine whether administration of a specific Therapeutic is indicated include *in vitro* cell culture assays in which appropriate cells from a cell line or cells cultured from a patient having a particular disease or disorder are exposed to or otherwise administered a Therapeutic, and the effect of the Therapeutic on the cells is observed.

Where the Therapeutic is a modified immunoglobulin that recognizes a cancer or tumor antigen, the potential efficacy of the modified immunoglobulin may be assayed by contacting the Therapeutic to cultured cells (either from a patient or cultured cell line) and then assaying for cell survival or growth using any method known in the art, for example, cell proliferation can be assayed by measuring ^{3}H-thymidine incorporation, by direct cell count, by detecting changes in transcriptional activity of known genes such as proto-oncogens *e.g.*, *fos*, *myc*) or cell cycle markers; cell viability can be assessed by trypan blue staining, differentiation can be assessed visually based on changes in morphology, etc.

Where the Therapeutic is a modified antibody that recognizes an antigen of an infectious disease agent or a cellular receptor for an infectious disease agent, the potential efficacy of the antibody may be assayed by contacting the Therapeutic to cultured cells (either from a patient or cultured cell line) that are infected with the infectious disease agent and then assaying the cells for reduction in the infectious disease agent or for reduction in physiological indicators of infection with the infectious disease agent. Alternatively, the Therapeutic may be assayed by contacting the Therapeutic to cells (either cultured from a patient or from a cultured cell line) that are susceptible to infection by the infectious disease agent but that are not infected with the infectious disease agent, exposing the cells to the infectious disease agent, and then determining whether the infection rate of cells contacted with the Therapeutic was lower than the infection rate of cells not so contacted with the Therapeutic. Infection of cells with an infectious disease agent may be assayed by any method known in the art.

Wherr the Therapeutic is a modified immunoglobulin specific for a particular ligand or receptor, the potential efficacy of the modified immunoglobulin may be tested by contacting the Therapeutic to cultured cells (either from a patient or cultured cell line) that express the receptor member of the binding pair and determining whether the Therapeutic prevents ligand binding to the receptor and/or receptor signaling or if the Therapeutic stimulates receptor signaling. These indicators can be measured by any method known in the art for measuring ligand-receptor binding and receptor signaling (*e.g.*, as exemplified in Section 6).

The Therapeutics may also be tested for efficacy in appropriate animal models, and in clinical trials, in humans. The efficacy of the Therapeutic may be determined by any method in the art, for example, after administration of the Therapeutic to the animal model or to the humann subject, the animal or human subject is evaluated for any indicator of the disease or disorder that the Therapeutic is intended to treat. For example, the efficacy of the Therapeutic can be assessed by measuring the level of the molecule against which the

modified antibody is directed in the animal model or human subject at suitable time intervals before, during, or after therapy. Any change or absence of change in the amount of the molecule can be identified and correlated with the effect of the treatment on the subject. The level of the molecule can be determined by any method known in the art, *e.g.*, by any of the immunoassay methods described in Section 5.5, *supra*, or 5.7, *infra*.

In other aspects, the modified antibodies may be tested for efficacy by monitoring the subject for improvement or recovery from the particular disease or condition associated with the molecule against which the synthetic modified antibody is directed. When the modified antibody is directed against a tumor or a cancer antigen, the progress of the particular tumor or cancer may be followed by any diagnostic or screening method known for monitoring cancer or a tumor. For example, but not by way of limitation, the process of the cancer or tumor may be monitored by assaying the levels of the particular cancer or tumor antigen (or another antigen associated with the particular cancer or tumor) either in the serum of the subject or by injecting a labeled antibody specific for the antigen. Additionally, other imaging techniques, such as computer tomographic (CT) scan or sonograms, or any other imaging method, may be used to monitor the progression of the cancer or tumor. Biopsies may also be performed. Before carrying out such trials in humans, the tests for efficacy of the modified immunoglobulins can be performed in animal models of the particular cancer or tumor.

Where the Therapeutic is specific for an antigen of an infectious disease agent or a cellular receptor of an infectious disease agent, the efficacy of the modified antibody can be assayed by administering the modified antibody to a subject (either a human subject or an animal model for the disease) and then monitoring either the levels of the particular infectious disease agent or symptoms of the particular infectious disease. The levels of the infectious disease agent may be determined by any method known in the art, for assaying the levels of an infectious disease agent, *e.g.*, the viral titer, in the case of a virus, or bacterial levels (for example, by culturing of a sample from the patient), etc. The levels of the infectious disease agent may also be determined by measuring the levels of the antigen against which the modified immunoglobulin was directed. A decrease in the levels of the infectious disease agent or an amelioration of the symptoms of the infectious disease indicates that the modified antibody is effective.

Where the therapeutic is administered as a vaccine, the immunopotency of a vaccine formulation containing the modified antibody of the invention can be determined by monitoring the anti-idiotypic response of test animals following immunization with the vaccine. Generation of a humoral response may be taken as an indication of a generalized

immune response, other components of which, particularly cell-mediated immunity, may be important for protection against a disease. Test animals may include mice, rabbits, chimpanzees and eventually human subjects. A vaccine made in this invention can be made to infect chimpanzees experimentally. However, since chimpanzees are a protected species, the antibody response to a vaccine of the invention can first be studied in a number of smaller, less expensive animals, with the goal of finding one or two best candidate immunoglobulin molecules or best combinations of immunoglobulin molecules to use in chimpanzee efficacy studies.

The immune response of the test subjects can be analyzed by various approaches such as the reactivity of the resultant immune serum to antibodies, as assayed by known techniques, *e.g.*, enzyme linked immunosorbent assay (ELISA), immunoblots, radioimmunoprecipitations, etc.; or protection from infection and/or attenuation of disease symptoms in immunized hosts.

As one example of suitable animal testing, the vaccine composition of the invention may be tested in rabbits for the ability to induce an anti-idiotypic response to the modified immunoglobulin molecule. For example, male specific-pathogen-free (SPF) young adult New Zealand White rabbits may be used. The test group of rabbits each receives an effective amount of the vaccine. A control group of rabbits receives an injection in 1 mM Tris-HCl pH 9.0 of the vaccine containing a naturally occurring antibody. Blood samples may be drawn from the rabbits every one or two weeks, and serum analyzed for anti-idiotypic antibodies to the modified immunoglobulin molecule and anti-anti-idiotypic antibodies specific for the antigen against which the modified antibody was directed using, *e.g.*, by a radioimmunoassay (Abbott Laboratories). The presence of anti-idiotypic antibodies may be assayed using an ELISA. Because rabbits may give a variable response due to their outbred nature, it may also be useful to test the vaccines in mice.

5.7. ASSAYS OF THE MODIFIED IMMUNOGLOBULINS

After constructing an immunoglobulin having one or more CDRs containing a binding site for a particular molecule, any binding assay known in the art can be used to assess the binding between the resulting modified antibody and the particular molecule. These assays may also be performed to select antibodies that exhibit a higher affinity or specificity for the particular antigen.

For example, but not by way of limitation, binding of the modified antibody to the particular molecule can be assayed using various immunoassays known in the art including but not limited to, competitive and non-competitive assay systems using techniques such as

radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitin reactions, immonodiffusion assays, *in situ* immunoassays (using colloidal gold, enzyme or radioisotope lables, for example), western blots, precipitation reactions, agglutination assays (*e.g.*, gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labelled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

An *in vitro* assay system useful in assessing the binding of the modified antibody to its target molecule is described below. Briefly, a reaction mixture of the modified antibody and the test sample is incubated under conditions and for a time sufficient to allow the two components to interact with, *e.g.*, bind to each other, thus forming a complex, which can represent a transient complex, which can be removed and/or detected in the reaction mixture. These assays can be conducted in a variety of ways. For example, one method to conduct such an assay would involve anchoring the modified antibody or the test substance onto a solid phase and detecting the antibody/molecule complexes anchored on the solid phase at the end of the reaction. In one embodiment of such a method, the modified antibody may be labeled, either directly or indirectly, and the test sample be anchored onto a solid surface. In practice, microtiter plates may conveniently be utilized as the solid phase. The anchored component may be immobilized by non-covalent or covalent attachments. Non-covalent attachment may be accomplished by simply coating the solid surface with a solution of the test sample and drying.

In order to conduct the assay, the nonimmobilized component is added to the coated surface containing the anchored component. After the reaction is complete, unreacted components are removed (*e.g.*, by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the previcusly nonimmobilized component is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously nonimmobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface.

Alternatively, a reaction can be conducted in a liquid phase, the reaction products separated from unreacted components, and complexes detected.

5.8. TRANSGENIC ANIMALS

The invention also provides animals that are transgenic for (*i.e.*, contain a nucleic acid encoding) a modified immunoglobulin of the invention (or a functional fragment thereof). Animals of any species, including, but not limited to, mice, rats, rabbits, guinea pigs, sheep, pigs, micro-pigs, goats, and non-human primates, *e.g.*, baboons, monkeys, and chimpanzees, may be used to generate transgenic animals of the invention.

Accordingly, in specific embodiments, the invention provides recombinant non-human animals containing a recombinant nucleic acid that contains a nucleotide sequence encoding a modified immunoglobulin of the invention, in particular, a recombinant non-human animal that is transgenic for a nucleic acid encoding a modified antibody that immunospecifically binds a cancer or tumor antigen or that is transgenic for a nucleic acid encoding a modified antibody that immunospecifically binds an antigen of an infectious disease agent or a cellular receptor of an infectious disease agent.

Any technique known in the art may be used to introduce the antibody transgene into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to pronuclear microinjection (Hoppe and Wagner, 1989, U.S. Pat. No. 4,873,191); retrovirus mediated gene transfer into germ lines (Van der Putten et al., 1985, *Proc. Natl. Acad. Sci. USA* 82:6148-6152); gene targeting in embryonic stem cells (Thompson et al., 1989, *Cell* 56:313-321); electroporation of embryos (Lo, 1983, *Mol Cell. Biol.* 3:1803-1814); and sperm-mediated gene transfer (Lavitrano et al., 1989, *Cell* 57:717-723); etc. For a review of such Techniques, see Gordon, 1989, *Transgenic Animals, Intl. Rev. Cytol.* 115:171-229, which is incorporated by reference herein in its entirety.

The present invention provides for transgenic animals that carry the nucleotide sequence encoding the modified antibody as transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, *i.e.*, mosaic animals. The transgene may be integrated as a single transgene or in concatamers, *e.g.*, head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko et al., 1992, *Proc. Natl. Acad. Sci. USA* 89:6232-6236). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When it is desired that the nucleotide encoding the synthetic antibody transgene be integrated into the chromosomal site of the endogenous immunoglobulin, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous immunoglobulin are designed for the purpose of integrating, via homologous recombination

with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous immunoglobulin gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous immunoglobulin in only that cell type, by following, for example, the teaching of Gu et al. (Gu et al., 1994, *Science* 265:103-106). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

Methods for the production of single-copy transgenic animals with chosen sites of integration are also well known to those of skill in the art (see, for example, Bronson et al., 1996, *Proc. Natl. Acad. Sci. USA* 93:9067-9072).

Once transgenic animals have been generated, the expression of the recombinant antibody gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to assay whether integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include but are not limited to Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and RT-PCR. Samples of gene-expressing tissue, may also be evaluated immunocytochemically using antibodies specific for the antibody transgene product.

6. EXAMPLE: BRADYKININ-CONTAINING SYNTHETIC MODIFIED ANTIBODIES

This example describes the construction of synthetic modified antibodies that immunospecifically bind to the bradykinin receptor (BR). The bradykinin receptor binds to a native ligand called bradykinin. The BR-bradykinin interaction is one example of a binding pair that may be used in the methods of the invention. The BR-bradykinin interaction occurs when amino acids in bradykinin, known as the binding site, contact the bradykinin receptor. The synthetic modified antibodies of this example, contain amino acids derived from the bradykinin binding site. These synthetic modified antibodies, therefore, mimic the bradykinin ligand and predictably bind to the bradykinin receptor (BR). Six synthetic modified antibodies containing bradykinin sequences were constructed and demonstrated to bind BR as constructed as described below.

The strategy for producing synthetic modified antibodies containing bradykinin binding sequences is outlined as follows:

1) using oligonucleotides, a variable region gene was engineered to contain a CDR with a bradykinin binding sequence;

2) the engineered variable region gene was then inserted into a mammalian expression vectors containing the appropriate constant regions;

3) a vector containing both light and heavy chains was transfected into a mammalian cell and the synthetic modified antibody was expressed; and

4) the synthetic modified antibodies were assayed for BR binding.

6.1. CONSTRUCTION OF THE VARIABLE REGION GENE CONTAINING BRADYKININ BINDING SITE

In order to construct the variable region gene encoding a CDR containing the binding site of bradykinin, the following strategy was performed.

First, single strand oligonucleotides were annealed to create cohesive double stranded DNA fragments (as diagramed in Figure 5, Step 1; see also, Kutemeier et al., 1994 *BioTechniques* 17:242). Specifically, oligonucleotides of about 80 bases in length corresponding to the sequences of interest with 20 base overlapping regions were synthesized using automated techniques of GenoSys Biotech Inc. The specific sequences of these oligonucleotides are presented in Figures 6A and B (for construction of the light and heavy chain variable regions, respectively). Figure 6A lists the sequences of the oligonucleotides used in engineering the light chain variable region genes containing a bradykinin binding sequence. Figure 6B lists the sequences of the oligonucleotides used in engineering the heavy chain variable region genes containing a bradykinin binding sequence. The combination of oligos used to engineer the six bradykinin CDRs (BKCDR1, BKCDR2, BKCDR3, BKCDR4, BKCDR5, BKCDR6) as well as the two consensus region (ConVL1 and ConVH1) are listed in Table 5.

Table 5 Oligonucleotides used in engineering synthetic modified antibodies with bradykinin sequence.

Name	Oligo1	Oligo 2	Oligo 3	Oligo 4	Oligo 5	Oligo 6	Oligo 7	Oligo 8	Oligo 9	Oligo10	Oligo11	Oligo12
ConV1	L1	BKLC1	BKLC2	BKLC3	BKLC4	BKLC5	BKLC6	BKLC7	BKLC8	BKLC9	BKLC10	L2
BKCDR1	L1	BKLC1	BKLCDR12	BKLC3	BKLC4	BKLC5	BKLC6	BKLC7	BKLC8	BKLCDR19	BKLC10	L2
BKCDR2	L1	BKLC1	BKLC2	BKLCDR23	BKLC4	BKLC5	BKLC6	BKLC7	BKLCDR28	BKLC9	BKLC10	L2
BKCDR3	L1	BKLC1	BKLC2	BKLC3	BKLC4	BKLCDR35	BKLCDR36	BKLC7	BKLC8	BKLC9	BKLC10	L2
ConVH1	BKHC1	BKHC2	BKHC3	BKHC4	BKHC5	BKHC6	BKHC7	BKHC8	BKHC9	BKHC10		
BKCDR4	BKHC1	BKHDR42	BKHDR43	BKHC4	BKHC5	BKHC6	BKHC7	BKHC8	BKHDR49	BKHC10		
BKCDR5	BKHC1	BKHC2	BKHDR53	BKHC4	BKHC5	BKHC6	BKHC7	BKHDR58	BKHC9	BKHC10		
BKCDR6	BKHC1	BKHC2	BKHC3	BKHC4	BKHC5	BKHC6	BKHC7	BKHC8	BKHC9	BKHC10		

In order to combine the oligos into the desired gene, groups of 10 or 12 oligos were used to engineer a variable region gene as described below. Each oligonucleotide was 5' phosphorylated as follows: 25 µl of each oligo was incubated for 1 hour in the presence of T4 polynucleotide kinase and 50 mM ATP at 37°C. The reactions were stopped by heating for 5 minutes at 70°C followed by ethanol precipitation. Once phosphorylated, complementary oligonucleotides (oligo 1 + oligo 10, oligo 2 + oligo 9, oligo 3 + oligo 8, oligo 4 + oligo 7, oligo 5 + oligo 6) as shown in Figure 5, were then mixed in sterile microcentrifuge tubes and annealed by heating the tube in a water bath at 65°C for 5 minutes followed by cooling at room temperature for 30 minutes. Annealing resulted in short double strand DNA fragments with cohesive ends.

Next, the cohesive double stand DNA fragments were ligated into longer strands (Figure 5, Steps 2-4), until the engineered variable region gene was assembled. Specifically, cohesive double strand DNA fragments were ligated in the presence of T4 DNA ligase and 10 mM ATP for 2 hours in a water bath maintained at 16°C. Annealed oligo 1/10 was mixed with annealed oligo 2/9, and annealed oligo 3/8 was mixed with annealed oligo 4/7. The resulting oligos were labeled oligo 1/10/2/9 and oligo 3/8/4/7. Next, oligo 3/8/4/7 was ligated to oligo 5/6. The resulting oligo 3/8/4/7/5/6 was then ligated to oligo 1/10/2/9 which resulted in a full length variable region gene.

Alternatively, when a group of 12 oligos were used, the order of addition was oligo numbers 1+12 = 1/12, 2+11=2/11, 3+10=3/10, 4+9=4/9, 5+8=5/8, 6+7=6/7, 1/12+2/11=1/12/2/11, 3/10+4/9=3/10/4/9, 5/8+6/7=5/8/6/7, 1/12/2/11+3/10/4/9 = 1/12/2/11/3/10/4/9, 1/12/2/11/3/10/4/9+5/8/6/7= full length variable region gene. Eight variable region genes were constructed by this method. Four genes were light chain variable region and four genes were heavy chain variable region. The engineered light chain genes included ConVL1, a consensus light chain variable region without a bradykinin sequence; BKCDR1, a light chain variable region containing bradykinin sequence in CDR1; BKCDR2, a light chain variable region containing bradykinin sequence in CDR2; and BKCDR3, a light chain variable region containing bradykinin sequence in CDR3. The engineered heavy chain variable region genes included ConVH1, a consensus heavy chain variable region without a bradykinin sequence; BKCDR4, a heavy chain variable region containing bradykinin sequence in CDR4; BKCDR5, a heavy chain variable region containing bradykinin sequence in CDR5; and BKCDR6, a heavy chain variable region containing bradykinin sequence in CDR6. The sequences of the eight engineered variable region genes is shown in Figures 4A to 4F.

Each one of the engineered gene made by combining oligonucleotides was treated as follows:

The resulting engineered variable region gene was purified by gel electrophoresis. To remove unligated excess of oligonucleotides and other incomplete DNA fragments, ligated product was run on 1% low melting agarose gel at constant 110 V for 2 hours. The major band containing full length DNA product was cut out and placed in a sterile 1.5 ml centrifuge tube. To release the DNA from the agarose, the gel slice was digested with ß-Agrase I at 40°C for 3 hours. The DNA was recovered by precipitation with 0.3 M NaOAc and isopropanol at –20°C for 1 hour followed by centrifugation at 12,000 rpm for 15 minutes. The purified DNA pellet was resuspended in 50 µl of TE buffer, pH 8.0. The engineered variable region gene was then amplified by PCR. Specifically, 100 ng of the engineered variable region gene was mixed with 25mM dNTPs, 200 ng of primers and 5 U of high fidelity thermostable Pfu DNA polymerase in buffer. DNA was amplified for 28 cycles. Resulting PCR product was analyzed on 1% agarose gel.

Each purified DNA corresponding to the engineered variable region genes was subsequently inserted into the pUC19 bacterial vector. pUC19, is a 2686 base pair, a high copy number *E. coli* plasmid vector containing a 54 base pair polylinker cloning site in lacZ and an Amp selection marker. In order to prepare the vector for insertion of the engineered variable region gene, 10 µg of pUC19 was linearized with *Hinc II* (50 U) for 3 hours at 37°C resulting in a vector with blunt end sequence 5' GTC. To prevent self re-ligation, linear vector DNA was dephosphorylated with 25 U of calf intestine alkaline phosphatase (CIP) for 1 hour at 37°C. In order to insert the engineered variable region gene into the pUC19 vector, approximately 0.5 µg of dephosphorylated linear vector DNA was mixed with 3 µg of phosphorylated variable region gene in the presence of T4 DNA ligase (1000 U), and incubated at 16°C for 12 hours.

The bacterial vector containing the engineered variable region gene was then used to transform bacterial cells. Specifically, freshly prepared competent DH5-α cells, 50 µl, were mixed with 1 µg of pUC19 containing the engineered variable region gene and transferred to an electroporation cuvette (0.2 cm gap; Bio-Rad). Each cuvette was pulsed at 2.5 kV/200 ohm/25 µF in an electroporator (Bio-Rad Gene Pulser). Immediately thereafter, 1 ml of SOC media was added to each cuvette and cells were allowed to recover for 1 hour at 37°C in centrifuge tubes. An aliquot of cells from each transformation was removed, diluted 1:100, then 100 µl plated onto LB plates containing ampicillin (Amp 40 µg/ml). The plates were incubated at 37°C overnight due to the presence of the Amp marker. Only transformants containing pUC19 vector grew on LB/Amp plates.

A single transformant colony was picked and grown overnight in a 3 ml LB/Amp sterile glass tube with constant shaking at 37°C. The plasmid DNA was isolated using Easy Prep columns (Pharmacia Biotech.) and suspended in 100 µl of TE buffer, pH 7.5. To confirm the presence of gene insert in pUC19, 25 µl of plasmid DNA from each colony was digested with *Hinc II* restriction endonuclease for 1 hour at 37°C, and was analyzed on a 1% agarose gel. By this method plasmid DNA containing gene insert was resistant to enzyme cleavage due to loss of restriction site (5'..GTCGAC.. 3') and migrated as closed circular (CC) DNA, while those plasmids without insert were cleaved and migrated as linear (L) double strand DNA fragment on gel.

In order to confirm correct gene sequences of the engineered variable region genes and to eliminate the possibility of unwanted mutations generated during the construction procedure, DNA sequencing was performed using M13/pUC reverse primer (5'AACAGCTATGACCATG 3') for the clones as well as PCR gene products using 5' end 20 base primer (5' GAATTCATGGCTTG GGTGTG 3') on automated ABI 377 DNA Sequencer. All clones were confirmed to contain correct sequences.

Six engineered variable region genes that contained bradykinin sequence were constructed by the methods of this example. Shown in Table 6 is the name of the synthetic modified antibody and the location corresponding bradykinin binding sequence within the variable region gene. For example, the synthetic antibody named hAbBKCDR1 contained bradykinin binding sequence (BK) in the CDR1 of the variable region light chain gene (V_L). This synthetic antibody had a consensus sequence (con) in the variable region heavy chain gene (V_H).

Table 6. Bradykinin-containing synthetic modified antibodies

Name of Synthetic Modified Antibody	V_L	V_H
hAbBKCDR1	BKCDR1	ConVH1
hAbBKCDR2	BKCDR2	ConVH1
hAbBKCDR3	BKCDR3	ConVH1
hAbBKCDR4	ConVL1	BKCDR4
hAbBKCDR5	ConVL1	BKCDR5
hAbBKCDR6	ConVL1	BKCDR6

The amino acid sequences corresponding to variable regions of each of the six synthetic modified antibodies of this example are listed in Table 7. CDRs are shown in

bold. The Bradykinin binding site amino acids are: ArgProProGlyPheSerProPheArg and are indicated in the CDRs by underlines. Table 5 also illustrates the consensus sequence of a human kappa light chain V_L subgroup I and human heavy chain V_H subgroup I gene. In cases where the consensus CDR was too short to include the complete bradykinin binding site sequence, the amino terminal residues from the bradykinin binding site were deleted since the carboxyterminal residues were known to be more important in receptor binding (Stewart and Vavrek, Chemistry of peptide B2 bradykinin antagonists, pp. 5196, Burch, R.M., editor, Bradykinin Antagonists, Basic and Clinical Research, New York: Marcel Dekker, 1991; hereby incorporated by reference).

Table 7. Amino acid sequences of engineered variable region genes.

Human kappa Light Chain V_L Subgroup (Kabat et al, 1991)

Amino Acid	Region	Consensus	BKCDR1	BKCDR2	BKCDR3
1	FR1	Asp	Asp	Asp	Asp
2			Ile	Ile	Ile
3		Gln	Gln	Gln	Gln
4		Met	Met	Met	Met
5		Thr	Thr	Thr	Thr
6		Gln	Gln	Gln	Gln
7		Ser	Ser	Ser	Ser
8		Pro	Pro	Pro	Pro
9		Ser	Ser	Ser	Ser
10		Ser	Ser	Ser	Ser
11		Leu	Leu	Leu	Leu
12		Ser	Ser	Ser	Ser
13		Ala	Ala	Ala	Ala
14		Ser	Ser	Ser	Ser
15		Val	Val	Val	Val
16		Gly	Gly	Gly	Gly
17		Asp	Asp	Asp	Asp
18		Arg	Arg	Arg	Arg
19		Val	Val	Val	Val
20		Thr	Thr	Thr	Thr
21		Ile	Ile	Ile	Ile
22		Thr	Thr	Thr	Thr
23		Cys	Cys	Cys	Cys
24	CDR1	**Arg**	**Arg**	**Arg**	**Arg**
25		**Ala**	**Pro**	**Ala**	**Ala**
26		**Ser**	**Pro**	**Ser**	**Ser**
27	(A-F)	**Gln**	**Gly**	**Gln**	**Gln**
28		**Ser**	**Phe**	**Ser**	**Ser**
29		**Ile**	**Ser**	**Ile**	**Ile**

Amino Acid	Region	Consensus	BKCDR1	BKCDR2	BKCDR3
30		**Ser**	**Pro**	**Ser**	**Ser**
31		**Asn**	**Phe**	**Asn**	**Asn**
32		**Tyr**	**Arg**	**Tyr**	**Tyr**
33		**Leu**	**Leu**	**Leu**	**Leu**
34		**Ala**	**Ala**	**Ala**	**Ala**
35	FR2	Trp	Trp	Trp	Trp
36		Tyr	Tyr	Tyr	Tyr
37		Gln	Gln	Gln	Gln
38		Gln	Gln	Gln	Gln
39		Lys	Lys	Lys	Lys
40		Pro	Pro	Pro	Pro
41		Gly	Gly	Gly	Gly
42		Lys	Lys	Lys	Lys
43		Ala	Ala	Ala	Ala
44		Pro	Pro	Pro	Pro
45		Lys	Lys	Lys	Lys
46		Leu	Leu	Leu	Leu
47		Leu	Leu	Leu	Leu
48		Ile	Ile	Ile	Ile
49		Tyr	Tyr	Tyr	Tyr
50	**CDR2**	**Ala**	**Ala**	**Pro**	**Ala**
51		**Ala**	**Ala**	**Gly**	**Ala**
52		**Ser**	**Ser**	**Phe**	**Ser**
53		**Ser**	**Ser**	**Ser**	**Ser**
54		**Leu**	**Leu**	**Pro**	**Leu**
55		**Glu**	**Glu**	**Phe**	**Glu**
56		**Ser**	**Ser**	**Arg**	**Ser**
57	FR3	Gly	Gly	Gly	Gly
58		Val	Val	Val	Val
59		Pro	Pro	Pro	Pro
60		Ser	Ser	Ser	Ser
61		Arg	Arg	Arg	Arg
62		Phe	Phe	Phe	Phe
63		Ser	Ser	Ser	Ser
64		Gly	Gly	Gly	Gly
65		Ser	Ser	Ser	Ser
66		Gly	Gly	Gly	Gly
67		Ser	Ser	Ser	Ser
68		Gly	Gly	Gly	Gly
69		Thr	Thr	Thr	Thr
70		Arg	Arg	Arg	Arg
71		Phe	Phe	Phe	Phe
72		Thr	Thr	Thr	Thr
73		Leu	Leu	Leu	Leu
74		Thr	Thr	Thr	Thr

Amino Acid	Region	Consensus	BKCDR1	BKCDR2	BKCDR3
75		Ile	Ile	Ile	Ile
76		Ser	Ser	Ser	Ser
77		Ser	Ser	Ser	Ser
78		Leu	Leu	Leu	Leu
79		Gln	Gln	Gln	Gln
80		Pro	Pro	Pro	Pro
81		Glu	Glu	Glu	Glu
82		Asp	Asp	Asp	Asp
83		Phe	Phe	Phe	Phe
84		Ala	Ala	Ala	Ala
85		Thr	Thr	Thr	Thr
86		Tyr	Tyr	Tyr	Tyr
87		Tyr	Tyr	Tyr	Tyr
88		Cys	Cys	Cys	Cys
89	**CDR3**	**Gln**	**Gln**	**Gln**	**Arg**
90		**Gln**	**Gln**	**Gln**	**Pro**
91		**Tyr**	**Tyr**	**Tyr**	**Pro**
92		**Asn**	**Asn**	**Asn**	**Gly**
93		**Ser**	**Ser**	**Ser**	**Phe**
94		**Leu**	**Leu**	**Leu**	**Ser**
95	**(A-F)**	**Pro**	**Pro**	**Pro**	**Pro**
96		**Trp**	**Trp**	**Trp**	**Phe**
97		**Thr**	**Thr**	**Thr**	**Arg**
98	FR4	Phe	Phe	Phe	Phe
99		Gly	Gly	Gly	Gly
100		Gin	Gin	Gin	Gin
101		Gly	Gly	Gly	Gly
102		Thr	Thr	Thr	Thr
103		Lys	Lys	Lys	Lys
104		Val	Val	Val	Val
105		Glu	Glu	Glu	Glu
106		Ile	Ile	Ile	Ile
107		Lys	Lys	Lys	Lys
108		Arg	Arg	Arg	Arg
109		Thr	Thr	Thr	Thr

Human Heavy Chain V_H Subgroup I (Kabat et al, 1991)

Amino Acid	Region	Consensus	BKCDR4	BKCDR5	BKCDR6
1	FR1	Gln	Gln	Gln	Gln
2		Val	Val	Val	Val
3		Gln	Gln	Gln	Gln
4		Leu	Leu	Leu	Leu
5		Val	Val	Val	Val

Amino Acid	Region	Consensus	BKCDR4	BKCDR5	BKCDR6
6		Gln	Gln	Gln	Gln
7		Ser	Ser	Ser	Ser
8		Gly	Gly	Gly	Gly
9		Ala	Ala	Ala	Ala
10		Glu	Glu	Glu	Glu
11		Val	Val	Val	Val
12		Lys	Lys	Lys	Lys
13		Lys	Lys	Lys	Lys
14		Pro	Pro	Pro	Pro
15		Gly	Gly	Gly	Gly
16		Ala	Ala	Ala	Ala
17		Ser	Ser	Ser	Ser
18		Val	Val	Val	Val
19		Lys	Lys	Lys	Lys
20		Val	Val	Val	Val
21		Ser	Ser	Ser	Ser
22		Cys	Cys	Cys	Cys
23		Lys	Lys	Lys	Lys
24		Ala	Ala	Ala	Ala
25		Ser	Ser	Ser	Ser
26		Gly	Gly	Gly	Gly
27		Tyr	Tyr	Tyr	Tyr
28		Thr	Thr	Thr	Thr
29		Phe	Phe	Phe	Phe
30		Thr	Thr	Thr	Thr
31	CDR4	**Ser**	**<u>Pro</u>**	**Ser**	**Ser**
32		**Tyr**	**<u>Gly</u>**	**Tyr**	**Tyr**
33		**Ala**	**<u>Phe</u>**	**Ala**	**Ala**
34		**Ile**	**<u>Ser</u>**	**Ile**	**Ile**
35	(A-B)	**Ser**	**<u>Pro</u>**	**Ser**	**Ser**
	35A	**Trp**	**<u>Phe</u>**	**Trp**	**Trp**
	35B	**Asn**	**<u>Arg</u>**	**Asn**	**Asn**
36	FR2	Trp	Trp	Trp	Trp
37		Val	Val	Val	Val
38		Arg	Arg	Arg	Arg
39		Gln	Gln	Gln	Gln
40		Ala	Ala	Ala	Ala
41		Pro	Pro	Pro	Pro
42		Gly	Gly	Gly	Gly
43		Gln	Gln	Gln	Gln
44		Gly	Gly	Gly	Gly
45		Leu	Leu	Leu	Leu
46		Glu	Glu	Glu	Glu
47		Trp	Trp	Trp	Trp
48		Met	Met	Met	Met

Amino Acid	Region	Consensus	BKCDR4	BKCDR5	BKCDR6
49		Gly	Gly	Gly	Gly
50	CDR5	**Trp**	**Trp**	**Trp**	**Trp**
51		Ile	Ile	Ile	Ile
52	(A-C)	Asn	Asn	Asn	Asn
53		Gly	Gly	Gly	Gly
54		Asn	Asn	Asn	Asn
39		Lys	Lys	Lys	Lys
40		Pro	Pro	Pro	Pro
41		Gly	Gly	Gly	Gly
42		Lys	Lys	Lys	Lys
43		Ala	Ala	Ala	Ala
44		Pro	Pro	Pro	Pro
45		Lys	Lys	Lys	Lys
46		Leu	Leu	Leu	Leu
47		Leu	Leu	Leu	Leu
48		Ile	Ile	Ile	Ile
49		Tyr	Tyr	Tyr	Tyr
50	CDR2	**Ala**	**Ala**	**Pro**	**Ala**
51		**Ala**	**Ala**	**Gly**	**Ala**
52		**Ser**	**Ser**	**Phe**	**Ser**
53		**Ser**	**Ser**	**Ser**	**Ser**
54		**Leu**	**Leu**	**Pro**	**Leu**
55		**Glu**	**Glu**	**Phe**	**Glu**
56		**Ser**	**Ser**	**Arg**	**Ser**
57	FR3	Gly	Gly	Gly	Gly
58		Val	Val	Val	Val
59		Pro	Pro	Pro	Pro
60		Ser	Ser	Ser	Ser
61		Arg	Arg	Arg	Arg
62		Phe	Phe	Phe	Phe
63		Ser	Ser	Ser	Ser
64		Gly	Gly	Gly	Gly
65		Ser	Ser	Ser	Ser
66		Gly	Gly	Gly	Gly
67		Ser	Ser	Ser	Ser
68		Gly	Gly	Gly	Gly
69		Thr	Thr	Thr	Thr
70		Arg	Arg	Arg	Arg
71		Phe	Phe	Phe	Phe
72		Thr	Thr	Thr	Thr
73		Leu	Leu	Leu	Leu
74		Thr	Thr	Thr	Thr
75		Ile	Ile	Ile	Ile
76		Ser	Ser	Ser	Ser
77		Ser	Ser	Ser	Ser

Amino Acid	Region	Consensus	BKCDR4	BKCDR5	BKCDR6
78		Leu	Leu	Leu	Leu
79		Gln	Gln	Gln	Gln
80		Pro	Pro	Pro	Pro
81		Glu	Glu	Glu	Glu
82		Asp	Asp	Asp	Asp
83		Phe	Phe	Phe	Phe
84		Ala	Ala	Ala	Ala
85		Thr	Thr	Thr	Thr
86		Tyr	Tyr	Tyr	Tyr
87		Tyr	Tyr	Tyr	Tyr
88		Cys	Cys	Cys	Cys
89	CDR3	**Gln**	**Gln**	**Gln**	**Arg**
90		**Gln**	**Gln**	**Gln**	**Pro**
55		**Gly**	**Gly**	**Pro**	**Gly**
56		**Asp**	**Asp**	**Pro**	**Asp**
57		**Thr**	**Thr**	**Gly**	**Thr**
58		**Asn**	**Asn**	**Phe**	**Asn**
59		**Tyr**	**Tyr**	**Ser**	**Tyr**
60		**Ala**	**Ala**	**Pro**	**Ala**
61		**Gln**	**Gln**	**Phe**	**Gln**
62		**Lys**	**Lys**	**Arg**	**Lys**
63		**Phe**	**Phe**	**Phe**	**Phe**
64		**Gln**	**Gln**	**Gln**	Gln
65		**Gly**	**Gly**	**Gly**	Gly
66	FR3	Arg	Arg	Arg	Arg
67		Val	Val	Val	Val
68		Thr	Thr	Thr	Thr
69		Ile	Ile	Ile	Ile
70		Thr	Thr	Thr	Thr
71		Ala	Ala	Ala	Ala
72		Asp	Asp	Asp	Asp
73		Thr	Thr	Thr	Thr
74		Ser	Ser	Ser	Ser
75		Thr	Thr	Thr	Thr
76		Ser	Ser	Ser	Ser
77		Thr	Thr	Thr	Thr
78		Ala	Ala	Ala	Ala
79		Tyr	Tyr	Tyr	Tyr
80		Met	Met	Met	Met
81		Glu	Glu	Glu	Glu
82	(A-C)	Leu	Leu	Leu	Leu
	82A	Ser	Ser	Ser	Ser
	82B	Ser	Ser	Ser	Ser
	82C	Leu	Leu	Leu	Leu

Amino Acid	Region	Consensus	BKCDR4	BKCDR5	BKCDR6
83		Arg	Arg	Arg	Arg
84		Ser	Ser	Ser	Ser
85		Glu	Glu	Glu	Glu
86		Asp	Asp	Asp	Asp
87		Thr	Thr	Thr	Thr
88		Ala	Ala	Ala	Ala
89		Val	Val	Val	Val
90		Tyr	Tyr	Tyr	Tyr
91		Tyr	Tyr	Tyr	Tyr
92		Cys	Cys	Cys	Cys
93		Ala	Ala	Ala	Ala
94		Arg	Arg	Arg	Arg
95	CDR6	**Ala**	**Ala**	**Ala**	**Ala**
96		**Pro**	**Pro**	**Pro**	**Pro**
97		**Gly**	**Gly**	**Gly**	**Gly**
98		**Tyr**	**Tyr**	**Tyr**	**Phe**
99		**Gly**	**Gly**	**Gly**	**Ser**
100	(A-K)	**Ser**	**Ser**	**Ser**	**Pro**
101		**Asp**	**Asp**	**Asp**	**Phe**
102		**Tyr**	**Tyr**	**Tyr**	**Arg**
103	FR4	Trp	Trp	Trp	Trp
91		**Tyr**	**Tyr**	**Pro**	**Pro**
92		**Asn**	**Asn**	**Asn**	**Gly**
93		**Ser**	**Ser**	**Ser**	**Phe**
94		**Leu**	**Leu**	**Leu**	**Ser**
95	(A-F)	**Pro**	**Pro**	**Pro**	**Pro**
96		**Trp**	**Trp**	**Trp**	**Phe**
97		**Thr**	**Thr**	**Thr**	**Arg**
98	FR4	Phe	Phe	Phe	Phe
99		Gly	Gly	Gly	Gly
100		Gln	Gln	Gln	Gln
101		Gly	Gly	Gly	Gly
102		Thr	Thr	Thr	Thr
103		Lys	Lys	Lys	Lys
104		Val	Val	Val	Val
105		Glu	Glu	Glu	Glu
106		Ile	Ile	Ile	Ile
107		Lys	Lys	Lys	Lys
108		Arg	Arg	Arg	Arg
109		Thr	Thr	Thr	Thr
104		Gly	Gly	Gly	Gly
105		Gln	Gln	Gln	Gln
106		Gly	Gly	Gly	Gly
107		Thr	Thr	Thr	Thr
108		Leu	Leu	Leu	Leu

Amino Acid	Region	Consensus	BKCDR4	BKCDR5	BKCDR6
109		Val	Val	Val	Val
110		Thr	Thr	Thr	Thr
111		Val	Val	Val	Val
112		Ser	Ser	Ser	Ser
113		Ser	Ser	Ser	Ser

6.2. INSERTION OF THE ENGINEERED VARIABLE REGION GENE INTO A MAMMALIAN EXPRESSION VECTOR

A complete antibody light chain has both a variable region and a constant region. A complete antibody heavy chain contains a variable region, a constant region, and a hinge region. In order to construct complete light chains and heavy chains, the modified variable region genes engineered above were then inserted into vectors containing the appropriate constant region. Engineered variable region genes with bradykinin sequence inserted into a light chain CDR, were inserted into the pMRRO10.1 vector (Figure 3A), which contains a human kappa light chain constant region. Insertion of the engineered light chain variable region into this vector gave a complete light chain sequence. Alternatively, engineered variable region genes with bradykinin sequence inserted into a heavy chain CDR, were inserted into the pGAMMA1 vector (Figure 3B),which contains the human IgG1 constant region and hinge region sequences. Insertion of the engineered heavy chain variable region gene into this vector resulted in a complete heavy chain sequence.

In order to engineer a mammalian vector encoding a complete antibody, both a complete heavy chain sequence and a light chain sequence were inserted into a single mammalian expression vector (Bebbington, C.R., 1991, In METHODS: A Companion to Methods in Enzymology, vol. 2, pp. 136-145). The resulting vector encoded both a light chain and heavy chain of antibody and was named pNEPuDGV (Figure 3C).

6.3. EXPRESSION OF SYNTHETIC MODIFIED ANTIBODIES IN MAMMALIAN CELLS

To examine the production of assembled antibodies the pNEPuDGV vector was transfected into COS cells. COS cells (an African green monkey kidney cell line, CV-1, transformed with an origin-defective SV40 virus) were used for short-term transient expression of the synthetic antibodies because of their capacity to replicate circular plasmids containing an SV40 origin of replication to very high copy number. The antibody expression vector was transfected into COS7 cells (obtained from the American Type Culture Collection) using calcium precipitation (Sullivan et al., *FEBS Lett.* 285:120-123,

1991). The transfected cells were grown in Dulbecco's modified Eagle's Medium and cultured for 72 hours after which supernatants containing the bradykinin-containing antibodies were collected. Supernatants from transfected COS cells were assayed using ELISA method for assembled IgG. The ELISA method involved capture of the samples and standards onto a 96-well plate coated with an anti-human IgG Fc. Bound assembled IgG was detected with an anti-human Kappa chain linked to horseradish peroxidase (HRP) and the substrate tetramethylbenzidine (TMB). Color development was proportional to the amount of assembled antibody present in the sample.

6.4. BRADYKININ-CONTAINING SYNTHETIC MODIFIED ANTIBODIES MIMIC BRADYKININ LIGANDS AND BIND TO BRADYKININ RECEPTOR

The synthetic modified antibodies engineered to contain bradykinin binding sequences were predicted to mimic the bradykinin ligand and bind the bradykinin receptor (BR). In order to confirm that these synthetic modified antibodies bound BR, the synthetic antibodies were assayed in a bradykinin receptor binding assay. The assay to examine synthetic antibody binding to BR was performed in the following manner. SV-T2 cells were transformed fibroblasts that express approximately 3,000 bradykinin receptors (BR) per cell. Stimulation of bradykinin receptors on SV-T2 cells leads to a rapid increase in PGE2 synthesis that is proportional to bradykinin binding. Therefore, PGE2 released into the medium is indicative of receptor binding.

As shown in Figure 7A, PGE2 synthesis was stimulated approximately four folds by the addition of 1 nM bradykinin (ligand). PGE2 synthesis was quantitated by ELISA. Also examined in Figure 7A was the receptor antagonist HOE-140. Addition of both HOE-140 and bradykinin or HOE-140 alone did not lead to PGE2 synthesis.

Further, as shown in Figure 7B, the expressed modified antibodies were assayed for their ability to bind and stimulate the bradykinin receptor. Medium from COS cells transfected with an antibody expression vector pNEPuDGV1 encoding either hABBKCDR3, hABBKCDR4, hABBKCDR5, or consensus was used to stimulate bradykinin receptors on SV-T2 cells. The synthetic antibodies having the variable chain regions BKCDR3 and BKCDR5 stimulated PGE2 synthesis in a dose dependent manner. BKCDR4, ConVH media alone, HOE-140 did not stimulate PGE2 synthesis (Figures 7B). The lack of PGE2 synthesis by cells exposed to BKCDR4 was likely attributed to the fact that the CDR4 consensus sequence was too short to accommodate the entire bradykinin binding sequence. Table 6 shows the comparison of consensus CDR amino acid sequences and BKCDR

sequences. The synthetic modified antibodies BKCDR3 and BKCDR5 were demonstrated to complete for receptor binding against the native ligand bradykinin. As shown in Figure 7C addition of bradykinin stimulated PGE2 synthesis four fold (second bar from left). Addition of either BKCDR3 or BKCDR 5 to cells prestimulated with native bradykinin inhibited the bradykinin-stimulated PGE2 synthesis.

Table 8

Consensus CDR3:	Gln Gln Tyr Asn Ser Leu Pro Trp Thr
BKCDR3:	Arg Pro Pro Gly Phe Ser Pro Phe Arg
Consensus CDR4:	Ser Tyr Ala Ile Ser Trp Asn
BKCDR4:	Pro Gly Phe Ser Pro Phe Arg
Consensus CDR5:	Trp Ile Asn Gly Asn Gly Asp Thr Asn Tyr Ala Gln Lys Phe Gln Gly
BKCDR5:	Trp Ile Asn Gly Arg Pro Pro Gly Phe Ser Pro Phe Arg Phe Gln Gly

Taken together, these results indicate that the modified antibodies containing the bradykinin binding site were able to bind the bradykinin receptor.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various references are cited herein, the disclosures of which are incorporated by reference in their entireties.

WHAT IS CLAIMED IS:

1. A modified immunoglobulin that immunospecifically binds a first member of a binding pair, which binding pair consists of said first member and a second member,

said immunoglobulin comprising a variable domain having at least one CDR containing a portion of said second member,

said portion containing a binding site for said first member and not being found naturally in the CDR,

said first member being a cancer antigen.

2. The modified immunoglobulin of claim 1 which is an antibody.

3. The modified immunoglobulin of claim 1 in which the first member is a tumor antigen.

4. The modified immunoglobulin of claim 3 in which said tumor antigen is polymorphic epithelial mucin antigen.

5. The modified immunoglobulin of claim 3 in which said tumor antigen is human colon carcinoma-associated protein antigen.

6. The modified immunoglobulin of claim 5, in which said portion has an amino acid sequence selected from the group consisting of Thr-Ala-Lys-Ala-Ser-Gln-Ser-Val-Ser-Asn-Asp-Val-Ala, Ile-Tyr-Tyr-Ala-Ser-Asn-Arg-Tyr-Thr, Phe-Ala-Gln-Gln-Asp-Tyr-Ser-Ser-Pro-Leu-Thr, Phe-Thr-Asn-Tyr-Gly-Met-Asn, Ala-Gly-Trp-Ile-Asn-Thr-Tyr-Thr-Gly-Glu-Pro-Thr-Tyr-Ala-Asp-Asp-Phe-Lys-Gly, and Ala-Arg-Ala-Tyr-Tyr-Gly-Lys-Tyr-Phe-Asp-Tyr.

7. The modified immunoglobulin of claim 3 in which said tumor antigen is a human colon carcinoma-associated carbohydrate antigen.

8. The modified immunoglobulin of claim 3 in which said tumor antigen is a human milk fat globule antigen.

9. The modified immunoglobulin of claim 8, in which said portion has the amino acid sequence selected from the group consisting of Ala-Tyr-Trp-Ile-Glu, Glu-Ile-Leu-Pro-Gly-Ser-Asn-Asn-Ser-Arg-Tyr-Asn-Glu-Lys-Phe-Lys-Gly, Ser-Glu-Asp-Ser-Ala-Val-Tyr-Tyr-Cys-Ser-Arg-Ser-Tyr-Asp-Phe-Ala-Trp-Phe-Ala-Tyr, Lys-Ser-Ser-Gln-Ser-Leu-Leu-Tyr-Ser-Ser-Asn-Gln-Lys-Ile-Tyr-Leu-Ala, Trp-Ala-Ser-Thr-Arg-Glu-Ser, and Gln-Gln-Tyr-Tyr-Arg-Tyr-Pro-Arg-Thr.

10. The modified immunoglobulin of claim 8, which further comprises a second CDR containing a portion of the second member having the amino acid sequence selected from the group consisting of: Ala-Tyr-Trp-Ile-Glu, Glu-Ile-Leu-Pro-Gly-Ser-Asn-Asn-Ser-Arg-Tyr-Asn-Glu-Lys-Phe-Lys-Gly, Ser-Glu-Asp-Ser-Ala-Val-Tyr-Tyr-Cys-Ser-Arg-Ser-Tyr-Asp-Phe-Ala-Trp-Phe-Ala-Tyr, Lys-Ser-Ser-Gln-Ser-Leu-Leu-Tyr-Ser-Ser-Asn-Gln-Lys-Ile-Tyr-Leu-Ala, Trp-Ala-Ser-Thr-Arg-Glu-Ser, and Gln-Gln-Tyr-Tyr-Arg-Tyr-Pro-Arg-Thr.

11. The modified immunoglobulin of claim 3 in which said tumor antigen is an antigen for a tumor of the breast, ovary, uterus, prostate, bladder, lung, skin, pancreas, colon, gastrointestinal, B lymphocyte or T lymphocyte.

12. The modified immunoglobulin of claim 1 in which said cancer antigen is selected from the group consisting of KS 1/4 pan-carcinoma antigen, ovarian carcinoma antigen, prostatic acid phosphate, prostate specific antigen, melanoma-associated antigen p97, melanoma antigen gp75, high molecular weight melanoma antigen, prostate specific membrane antigen, carcinoembryonic antigen, polymorphic epithelial mucin antigen, human milk fat globule antigen, colorectal tumor-associated antigen TAG-72, CO17-1A, GICA 19-9, CTA-1, LEA, Burkitt's lymphoma antigen-38.13, CD19, human B-lymphoma antigen-CD20, CD33, ganglioside GD2, ganglioside GD3, ganglioside GM2, ganglioside GM3, tumor-specific transplantation type of cell-surface antigen, oncofetal antigen-alpha-fetoprotein L6, human lung carcinoma antigen L20, human leukemia T cell antigen-Gp37, neoglycoprotein, sphingolipids, EGFR, HER2 antigen, polymorphic epithelial mucin, malignant human lymphocyte antigen-APO-1, I antigen M18, M39, SSEA-1, VEP8, VEP9, Myl, VIM-D5, $D_1$56-22, TRA-1-85, C14, F3, AH6, Y hapten, Le^y, TL5, FC10.2, gastric adenocarcinoma antigen, CO-514, NS-10, CO-43, MH2, 19.9 found in colon cancer, gastric cancer mucins, T_5A_7, R_{24}, 4.2, G_{D3}, D1.1, OFA-1, G_{M2}, OFA-2, G_{D2}, M1:22:25:8, SSEA-3, SSEA-4, and a Tcell receptor derived peptide.

13. A modified immunoglobulin that immunospecifically binds a first member of a binding pair, which binding pair consists of said first member and a second member,

said immunoglobulin comprising a variable domain having at least one CDR containing a portion of said second member,

said portion containing a binding site for said first member and not being found naturally in the CDR,

said first member being an antigen of an infectious disease agent.

14. The modified immunoglobulin of claim 13 which is an antibody.

15. The modified immunoglobulin of claim 13 in which said infectious disease agent is a bacterium.

16. The modified immunoglobulin of claim 13 in which said infectious disease agent is a virus.

17. The modified immunoglobulin of claim 13 in which said infectious disease agent is a parasite.

18. The modified immunoglobulin of claim 13 in which the antigen for the infectious disease agent is selected from the group consisting of a Brambell receptor, an antigen of HSV-2, an antigen of a gonnococcus, an antigen of Treponema pallidum, an antigen of Chlamydia trachomatis, or an antigen of human papillomavirus.

19. The modified immunoglobulin of claim 13 in which the antigen for the infectious disease agent is selected from group consisting of influenza virus hemagglutinin, human respiratory syncytial virus G glycoprotein, core protein of Dengue virus, matrix protein of Dengue virus, measles virus hemagglutinin, herpes simplex virus type 2 glycoprotein gB, poliovirus I VP1, envelope glycoproteins of HIV I, hepatitis B surface antigen, diptheria toxin, streptococcus 24M epitope, gonococcal pilin, pseudorabies virus g50, pseudorabies virus glycoprotein H, pseudorabies virus glycoprotein E, transmissible gastroenteritis glycoprotein 195, transmissible gastroenteritis matrix protein, swine rotavirus glycoprotein 38, swine parvovirus capsid protein, *Serpulina hydodysenteriae* protective antigen, bovine viral diarrhea glycoprotein 55, Newcastle disease virus hemagglutinin-neuraminidase, swine flu hemagglutinin, swine flu neuraminidase, infectious bovine

rhinotracheitis virus glycoprotein E, infectious laryngotracheitis virus glycoprotein G or glycoprotein I, a glycoprotein of La Crosse virus, neonatal calf diarrhea virus, hepatitis B virus core protein, hepatitis B virus surface antigen, equine influenza virus type A/Alaska 91 neuraminidase, equine influenza virus type A/Miami 63 neuraminidase, equine influenza virus type A/Kentucky 81 neuraminidase, equine herpesvirus type 1 glycoprotein B, equine herpesvirus type 1 glycoprotein D, bovine respiratory syncytial virus attachment protein, bovine respiratory syncytial virus fusion protein, bovine respiratory syncytial virus nucleocapsid protein, bovine parainfluenza virus type 3 fusion protein, bovine parainfluenza virus type 3 hemagglutinin neuraminidase, bovine viral diarrhea virus glycoprotein 48, and bovine diarrhea virus glycoprotein 53.

20. The modified immunoglobulin of claim 15 in which the infectious disease agent is selected from a group consisting of mycobacteria rickettsia, mycoplasma, Neisseria spp., Shigella spp. legionella, *Vibrio cholerae*, Streptococci, *corynebacteria diphtheriae*, *clostridium tetani*, *bordetella pertussis*, Haemophilus spp., Chlamydia spp., and *Escherichia coli*.

21. The modified immunoglobulin of claim 16 in which the infectious disease agent is selected from a group consisting of hepatitis type A, hepatitis type B, hepatitis type C, influenza, varicella, adenovirus, herpes simplex type I, herpes simplex type II, rinderpest, rhinovirus, echovirus, rotavirus, respiratory syncytial virus, papilloma virus, papova virus, cytomegalovirus, echinovirus, arbovirus, hantavirus, coxsachie virus, mumps virus, measles virus, rubella virus, polio virus, human immunodeficiency virus type I, human immunodeficiency virus type II, picornaviruses, enteroviruses, caliciviridae, Norwalk group of viruses, togaviruses, alphaviruses, flaviviruses, coronaviruses, rabies virus, Marburg viruses, ebola viruses, parainfluenza virus, orthomyxoviruses, bunyaviruses, arenaviruses, reoviruses, rotaviruses, orbiviruses, human T cell leukemia virus type I, human T cell leukemia virus type II, simian immunodeficiency virus, lentiviruses, polyomaviruses, parvoviruses, Epstein-Barr virus, human herpesvirus-6, cercopithecine herpes virus 1, and poxviruses.

22. The modified immunoglobulin of claim 17 in which the infectious disease agent is selected from a group consisting of plasmodia, eimeria, leishmania, kokzidioa, and trypanosoma, and fungi.

23. A modified immunoglobulin that immunospecifically binds a first member of a binding pair, which binding pair consists of said first member and a second member,

said immunoglobulin comprising a variable domain having at least one CDR containing a portion of said second member,

said portion containing a binding site for said first member, which binding site does not have the sequence Asn-Ala-Asn-Pro or Asn-Val-Asp-Pro and is not found naturally in the CDR,

said first member being a cellular receptor for an infectious disease agent.

24. The modified immunoglobulin of claim 23 which is an antibody.

25. The modified immunoglobulin of claim 23 in which said infectious disease agent is a bacterium.

26. The modified immunoglobulin of claim 23 in which said infectious disease agent is a virus.

27. The modified immunoglobulin of claim 23 in which said infectious disease agent is a parasite.

28. The modified immunoglobulin of claim 23 in which the cellular receptor is selected from a group consisting of LPV receptor, adenylate cyclase, BDV surface glycoproteins, N-acetyl-9-O-acetylneuraminic acid receptor, CD4', highly sulphated type heparin sulphate, p65, Gal alpha 1-4-Gal-containing isoreceptors, CD16b, integrin VLA-2 receptor, EV receptor, CD14, glycoconjugate receptors, alpha/beta T-cell receptor, decay-accelerating factor receptor, extracellular envelope glycoprotein receptor, immunoglobulin Fc receptor poxvirus M-T7, GALV receptor, CD14 receptor, Lewis(b) blood group antigen receptor, T-cell receptor, heparin sulphate glycoaminoglycans receptor, fibroblast growth factor receptor, CD11a, CD2, G-protein coupled receptor, CD4, heparin sulphate proteoglycan, annexin II, CD13 (aminopeptidase N), human aminopeptidase N receptor, hemagglutinin receptor, CR3 receptor, protein kinase receptor, galactose N-acetylgalactosamine-inhibitable lectin receptor, chemokine receptor, annexin I, actA protein, CD46 receptor, meningococcal virulence associated opa receptors, CD46 receptor, carcinoembryonic antigen family receptors, carcinoembryonic antigen family Bg1a receptor, gamma interferon receptor, glycoprotein gp70, rmc-1 receptor, human integrin receptor

alpha v beta 3, heparin sulphate proteoglycan receptor, CD66 receptor, integrin receptor, membrane cofactor protein, CD46, GM1, GM2, GM3, CD3, ceramide, hemagglutinin-neuraminidase protein, erythrocyte P antigen receptor, CD36 receptor, glycophorin A receptor, interferon gamma receptor, KDEL receptor, mucosal homing alpha4beta7 receptor, epidermal growth factor receptor, alpha5beta1 integrin protein, non-glycosylated J774 receptor, CXCR1-4 receptor, CCR1-5 receptor, CXCR3 receptor, CCR5 receptor, gp46 surface glycoprotein, TNFR p55 receptor, TNFp75 receptor, soluble interleukin-1 beta receptor.

29. A modified immunoglobulin that immunospecifically binds a first member of a ligand-receptor binding pair, which binding pair consists of said first member and a second member,

said immunoglobulin comprising a variable domain having at least one CDR containing a portion of said second member,

said portion containing a binding site for said first member and not being found naturally in the CDR.

30. The modified immunoglobulin of claim 29 which is an antibody.

31. The modified immunoglobulin of claim 29 in which said first member is a receptor.

32. The modified immunoglobulin of claim 29 in which said first member is a ligand.

33. The modified immunoglobulin of claim 29 in which said first member is a receptor agonist.

34. The modified immunoglobulin of claim 29 in which said first member is a receptor antagonist.

35. The modified immunoglobulin of claim 29 in which said first member is a bradykinin receptor.

36. The modified immunoglobulin of claim 35, wherein said portion consists of the amino acid sequence Arg-Pro-Pro-Gly-Phe-Gly-Phe-Ser-Pro-Phe-Arg.

37. The modified immunoglobulin of claim 31 in which said receptor is selected from a group consisting of an opioid receptor, a glucose transporter, a glutamate receptor, an orphanin receptor, erythropoietin receptor, insulin receptor, tyrosine kinase receptor, KIT stem cell factor receptor, nerve growth factor receptor, insulin-like growth factor receptor, granulocyte-colony stimulating factor receptor, somatotropin receptor, glial-derived neurotrophic factor receptor, gp39 receptor, G-protein receptor class and ß2-adrenergic receptor

38. The modified immunoglobulin of claim 30 in which said ligand is selected from a group consisting of cholecystokinin, galanin, IL-1, IL-2, IL-4, IL-5, IL-6, IL-11, a chemokine, leptin, a protease, neuropeptide Y, neurokinin-1, neurokinin-2, neurokinin-3, bombesin, gastrin, corticotropin releasing hormone, endothelin, melatonin, somatostatin, vasoactive intestinal peptide, epidermal growth factor, tumor necrosis factor, dopamine, and endothelin.

39. The modified immunoglobulin of claim 2, 14, 24, or 30, in which said antibody is of a type selected from the group consisting of IgG, IgE, IgM, IgD and IgA.

40. A fragment of the modified immunoglobulin of claim 2, 14, 24, or 30, in which said fragment can immunospecifically bind said first member.

41. The fragment of claim 40, in which said fragment is selected from the group consisting of a Fab, a $(Fab')_2$, a heavy chain dimer, a light chain dimer, and a Fv fragment.

42. The modified immunoglobulin of claim 2, 14, 24, or 30, in which said portion is an insertion within said CDR.

43. The modified immunoglobulin of claim 2, 14, 24, or 30, in which said portion replaces one or more amino acids of the CDR.

44. The modified immunoglobulin of claim 2, 14, 24, or 30, in which said CDR containing said portion is a germline CDR.

45. The modified immunoglobulin of claim 2, 14, 24, or 30, in which said CDR containing said portion is a non-germline CDR.

46. The modified immunoglobulin of claim 2, 14, 24 or 30, in which said portion is at least 4 amino acids.

47. The modified immunoglobulin of claim 2, 14, 24, or 30, in which said portion is in the range of 10-20 amino acids.

48. The modified immunoglobulin of claim 2, 14, 24, or 30, in which said CDR containing said portion contains no more than 25 amino acids.

49. The modified immunoglobulin of claim 2, 14, 24, or 30, in which said CDR containing said portion is the first CDR of the heavy chain variable region.

50. The modified immunoglobulin of claim 2, 14, 24, or 30, in which said CDR containing said portion is the second CDR of the heavy chain variable region.

51. The modified immunoglobulin of claim 2, 14, 24, or 30, in which said CDR containing said portion is the third CDR of the heavy chain variable region.

52. The modified immunoglobulin of claim 2, 14, 24 or 30, in which said CDR containing said portion is the first CDR of the light chain variable region.

53. The modified immunoglobulin of claim 2, 14, 24, or 30, in which said CDR containing said portion is the second CDR of the light chain variable region.

54. The modified immunoglobulin of claim 2, 14, 24, or 30, in which said CDR containing said portion is the third CDR of the light chain variable region.

55. The modified immunoglobulin of claim 2, 14, 24, or 30, in which more than one CDR contains a portion of said binding site.

56. The modified immunoglobulin of claim 2, 14, 24, or 30 in which a second CDR contains a second binding site for a molecule other than said first member.

57. The modified immunoglobulin of claim 56 in which said molecule other than said first member is a molecule on the surface of an immune cell.

58. The modified immunoglobulin of claim 57 in which said immune cell is a T cell, B cell, NK cell, K cell, TIL cell or neutrophil.

59. The modified immunoglobulin of claim 2, 14, 24, or 30 which has a higher specificity for said first member than a naturally occurring antibody that immunospecifically binds said first member.

60. The modified immunoglobulin of claim 2, 14, 24, or 30 which has a higher affinity for said first member than a naturally occurring antibody that immunospecifically binds said first member.

61. The modified immunoglobulin of claim 2, 14, 24, or 30 which exhibits a binding constant for said first member of at least $2x10^7M$.

62. The modified immunoglobulin of claim 2, 14, 24, or 30, wherein said antibody possesses an affinity constant for said first member of at least $2x10^7M$.

63. The modified immunoglobulin of claim 1, 13, 23,or 29 in which one or more cysteine residues in the variable region of said immunoglobulin that form a disulfide bond are substituted with one or more amino acid residues that do not have a sulfhydryl group.

64. The modified immunoglobulin of claim 63 in which at least one of said one or more cysteine residues is at position 23 or 88 of the light chain variable region.

65. The modified immunoglobulin of claim 63 in which at least one of said one or more cysteine residues is at position 23 or 92 of the heavy chain variable region.

66. The modified immunoglobulin of claim 63 which at least one of said amino acid residues that do not have a sulfhydryl group is an alanine.

67. A molecule comprising a variable domain that immunospecifically binds a first member of a binding pair, which binding pair consists of said first member and a second member,

said variable domain having at least one CDR containing a portion of said second member,

said portion containing a binding site for said first member and not being found naturally in the CDR,

said first member being a cancer antigen.

68. A molecule comprising a variable domain that immunospecifically binds a first member of a binding pair, which binding pair consists of said first member and a second member,

said variable domain having at least one CDR containing a portion of said second member and not being found naturally in the CDR,

said portion containing a binding site for said first member,

said first member being an antigen of an infectious disease agent.

69. A molecule comprising a variable domain that immunospecifically binds a first member of a binding pair, which binding pair consists of said first member and a second member,

said variable domain having at least one CDR containing a portion of said second member,

said portion containing a binding site for said first member which binding site does not have the sequence Asn-Ala-Asn-Pro or Asn-Val-Asp-Pro and is not found naturally in the CDR,

said first member being a cellular receptor for an infectious disease agent.

70. A molecule comprising a variable domain that immunospecifically binds a first member of a ligand-receptor binding pair, which binding pair consists of said first member and a second member,

said variable domain having at least one CDR containing a portion of said second member,

said portion containing a binding site for said first member and not being found naturally in the CDR.

71. The molecule of claim 67, 68, 69, or 70, in which said molecule is a single chain antibody.

72. The molecule of claim 67, 68, 69, or 70, which further comprises a constant domain.

73. The molecule of claim 72 in which the variable domain is from a mouse antibody, except for the CDR containing said portion, and the constant domain is from a human antibody.

74. The molecule of claim 72 in which the variable domain has framework regions from a human antibody and CDRs from a mouse antibody, except for the CDR containing said portion, and in which the constant domain is from a human antibody.

75. The molecule of claim 74 in which said variable domain has at least one of framework region having at least one amino acid change with respect to the naturally occurring framework region.

76. The molecule of claim 67, 68, 69, or 70, which is fused via a covalent bond to an immunostimulatory or growth enhancing factor or a functional fragment thereof.

77. The molecule of claim 76 where the immunostimulatory factor is chosen from the group consisting of interleukin-2, interleukin-4, interleukin-5, interleukin-6. interleukin-7, interleukin-10, interleukin-12, interleukin-15, G-colony stimulating factor, tumor necrosis factor, porin, interferon-gamma, NK cell antigen., and a cellular endocytosis recptor.

78. An isolated nucleic acid comprising a nucleotide sequence encoding the modified immunoglobulin of claim 1, 13, 23, or 29.

79. An isolated nucleic acid comprising a nucleotide sequence encoding the molecule of claim 67, 68, 69, or 70.

80. The isolated nucleic acid of claim 78 in which said nucleic acid is a vector.

81. The isolated nucleic acid of claim 79 in which said nucleic acid is a vector.

82. A cell containing the nucleic acid of claim 78, which nucleic acid is recombinant.

83. A cell containing the nucleic acid of claim 79, which nucleic acid is recombinant.

84. A recombinant non-human animal containing the nucleic acid of claim 78.

85. A recombinant non-human animal containing the nucleic acid of claim 79.

86. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of the modified immunoglobulin of claim 1, 13, 23 or 29; and a pharmaceutically acceptable carrier.

87. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of the molecule of claim 67, 68, 69 or 70; and a pharmaceutically acceptable carrier.

88. A pharmaceutical composition comprising of a therapeutically or prophylactically effective amount of the nucleic acid of claim 78; and a pharmaceutically acceptable carrier.

89. A pharmaceutical composition comprising of a therapeutically or prophylactically effective amount of the nucleic acid of claim 79; and a pharmaceutically acceptable carrier.

90. A pharmaceutical composition comprising of a therapeutically or prophylactically effective amount of the recombinant cell of claim 82; and a pharmaceutically acceptable carrier.

91. A pharmaceutical composition comprising of a therapeutically or prophylactically effective amount of the recombinant cell of claim 83; and a pharmaceutically acceptable carrier.

92. A vaccine composition comprising an amount of the modified immunoglobulin of claim 1, 13, 23, or 29 sufficient to induce an immune response; and a pharmaceutically acceptable carrier.

93. A vaccine composition comprising an amount of the molecule of claim 67, 68, 69, or 70 sufficient to induce an immune response; and a pharmaceutically acceptable carrier.

94. A vaccine composition comprising an amount of the modified immunoglobulin of claim 63 sufficient to induce an anti-idiotype response; and a pharmaceutically acceptable carrier.

95. A method for identifying or measuring or detecting a cancer antigen in a sample to be tested, which cancer antigen is a first member of a binding pair consisting of said first member and a second member, said method comprising the steps of:

(a) contacting the sample to be tested with a modified immunoglobulin that can immunospecifically bind to said cancer antigen, said modified immunoglobulin comprising a variable domain having at least one CDR containing a portion of said second member, which portion contains a binding site for said cancer antigen and is not found naturally in the CDR, under conditions such that immunospecific binding of said modified immunoglobulin to any of said cancer antigen in the sample can occur; and

(b) detecting any binding of said modified immunoglobulin to said cancer antigen that occurs;

wherein detection of binding of said modified immunoglobulin to said cancer antigen indicates the presence of said cancer antigen in said sample.

96. A method for identifying or measuring or detecting an antigen of an infectious disease agent in a sample to be tested, which antigen is a first member of a binding pair consisting of said first member and a second member, said method comprising the steps of:

(a) contacting the sample to be tested with a modified immunoglobulin that can immunospecifically bind to said antigen, said modified immunoglobulin comprising a variable domain having at least one CDR containing a portion of said second member, which portion contains a binding site for said antigen and is not

found naturally in the CDR, under conditions such that immunospecific binding of said modified immunoglobulin to any of said antigen in the sample can occur; and

(b) detecting any binding of said modified immunoglobulin to said antigen that occurs;

wherein detection of binding of said modified immunoglobulin to said antigen indicates the presence of said antigen in said sample.

97. A method for identifying or measuring or detecting a ligand in a sample to be tested, which ligand is a first member of a binding pair consisting of said first member and a second member, said method comprising the steps of:

(a) contacting the sample to be tested with a modified immunoglobulin that can inmmunospecifically bind to said ligand, said modified immunoglobulin comprising a variable domain having at least one CDR containing a portion of said second member, which portion contains a binding site for said ligand and is not found naturally in the CDR, under conditions such that immunospecific binding of said modified immunoglobulin to any of said ligand in the sample can occur; and

(a) detecting any binding of said modified immunoglobulin to said ligand that occurs;

wherein detection of binding of said modified immunoglobulin to said ligand indicates the presence of said ligand in said sample.

98. A method for identifying or measuring or detecting a receptor in a sample to be tested, which receptor is a first member of a binding pair consisting of said first member and a second member, said method comprising the steps of:

(a) contacting the sample to be tested with a modified immunoglobulin that can immunospecifically bind to said receptor, said modified immunoglobulin comprising a variable domain having at least one CDR containing a portion of said second member, which portion contains a binding site for said receptor and is not found naturally in the CDR, under conditions such that immunospecific binding of said modified immunoglobulin to any of said receptor in the sample can occur; and

(a) detecting any binding of said modified immunoglobulin to said receptor that occurs;

wherein detection of binding of said modified immunoglobulin to said receptor indicates the presence of said receptor in said sample.

99. A kit for the detection of a cancer antigen, which cancer antigen is a first member of a binding pair consisting of said first member and a second member, said kit comprising in a container:

(a) a modified immunoglobulin which can immunospecifically bind said cancer antigen, said immunoglobulin comprising a variable domain having at least one CDR containing a portion of said second member, said portion containing a binding site for said cancer antigen and not being found naturally in the CDR; and

(b) a means to detect binding of said cancer antigen to said immunoglobulin.

100. A kit for the detection of an antigen of an infectious disease agent, which antigen is a first member of a binding pair consisting of said first member and a second member, said kit comprising in a container:

(a) a modified immunoglobulin which can immunospecifically bind said antigen, said immunoglobulin comprising a variable domain having at least one CDR containing a portion of said second member, said portion containing a binding site for said antigen and not being found naturally in the CDR; and

(b) a means to detect binding of said antigen to said immunoglobulin.

101. A kit for the detection of a cellular receptor for an infectious disease agent, which cellular receptor is a first member of a binding pair consisting of said first member and a second member, said kit comprising in a container:

(a) a modified immunoglobulin which can immunospecifically bind said cellular receptor, said immunoglobulin comprising a variable domain having at least one CDR containing a portion of said second member, said portion containing a binding site for said cellular receptor, which binding site does not have the sequence Asn-Ala-Asn-Pro or Asn-Val-Asp-Pro and is not found naturally in the CDR; and

(b) a means to detect binding of said cellular receptor to said immunoglobulin.

102. A kit for the detection of an ligand, which is a first member of a binding pair consisting of said first member and a second member, said kit comprising in a container:

(a) a modified immunoglobulin which can immunospecifically bind said ligand, said immunoglobulin comprising a variable domain having at least one CDR containing a portion of said second member, said portion containing a binding site for said ligand and not being found naturally in the CDR; and

(b) a means to detect binding of said ligand to said immunoglobulin.

103. A kit for the detection of a receptor, which is a first member of a binding pair consisting of said first member and a second member, said kit comprising in a container:

(a) a modified immunoglobulin which can immunospecifically bind said receptor, said immunoglobulin comprising a variable domain having at least one CDR containing a portion of said second member, said portion containing a binding site for said receptor and not being found naturally in the CDR; and

(b) a means to detect binding of said receptor to said immunoglobulin.

104. A method of diagnosing or screening for the presence of or a predisposition for developing a cancer characterized by the increased presence of a cancer antigen, which is a first member of a binding pair consisting of said first member and a second member, said method comprising measuring in a subject the level of immunospecific binding of a modified immunoglobulin to a sample derived from the subject, in which said modified immunoglobulin immunospecifically binds said cancer antigen and in which said modified immunoglobulin comprises a variable domain having at least one CDR containing a portion of said second member, said portion containing a binding site for said cancer antigen and not being found naturally in the CDR, in which an increase in the level of said immunospecific binding, relative to the level of said immunospecific binding in an analogous sample from a subject not having the cancer or a predisposition for developing the cancer, indicates the presence of the cancer or a predisposition for developing the cancer.

105. A method of diagnosing or screening for the presence of an infectious disease agent, characterized by the presence of an antigen of said infectious disease agent, which antigen is a first member of a binding pair consisting of said first member and a second member, said method comprising measuring in a subject the level of immunospecific binding of a modified immunoglobulin to a sample derived from the subject, in which said modified immunoglobulin immunospecifically binds said antigen and in which said modified immunoglobulin comprises a variable domain having at least one CDR containing a portion of said second member, said portion containing a binding site for said antigen and not being found naturally in the CDR, in which an increase in the level of said immunospecific binding, relative to the level of said immunospecific binding in an

analogous sample from a subject not having the infectious disease agent, indicates the presence of said infectious disease agent.

106. A method of treating or preventing, in a subject in need of such treatment or prevention, a cancer characterized by the presence of a cancer antigen, which cancer antigen is a first member of a binding pair consisting of said first member and a second member and which cancer antigen is immunospecifically bound by a modified immunoglobulin, said immunoglobulin comprising a variable domain having at least one CDR containing a portion of said second member, said portion containing a binding site for said cancer antigen and not being found naturally in the CDR, such method comprising administering to the subject a therapeutically or prophylactically effective amount of said modified immunoglobulin.

107. A method of treating or preventing, in a subject in need of such treatment or prevention, an infectious disease characterized by the presence of an antigen of an infectious disease agent, which antigen is a first member of a binding pair consisting of said first member and a second member and which antigen is immunospecifically bound by a modified immunoglobulin, said immunoglobulin comprising a variable domain having at least one CDR containing a portion of said second member, said portion containing a binding site for said antigen and not being found naturally in the CDR, comprising administering to the subject a therapeutically or prophylactically effective amount of said modified immunoglobulin.

108. A method of treating or preventing, in a subject in need of such treatment or prevention, a disease caused by an infectious disease agent that binds to a cellular receptor, which cellular receptor is a first member of a binding pair consisting of said first member and a second member and which cellular receptor is immunospecifically bound by a modified immunoglobulin, said immunoglobulin comprising a variable domain having at least one CDR containing a portion of said second member, said portion containing a binding site for said cellular receptor, which binding site does not have the sequence Asn-Ala-Asn-Pro or Asn-Val-Asp-Pro and is not found naturally in the CDR, said method comprising administering to the subject a therapeutically or prophylactically effective amount of said modified immunoglobulin.

109. A method for modulating the activity of a first member of a binding pair, which binding pair consists of a first and a second member, said method comprising administering the modified immunoglobulin of claim 1, 13, 23 or 29.

110. A method of producing a modified immunoglobulin that immunospecifically binds a cancer antigen, which cancer antigen is a first member of a binding pair consisting of said first member and a second member, said modified immunoglobulin comprising a variable domain having at least one CDR containing a portion of said second member, said portion containing a binding site for said cancer antigen and not being found naturally in the CDR, said method comprising growing a recombinant cell containing a nucleic acid comprising a nucleotide sequence encoding the modified immunoglobulin such that the encoded modified immunoglobulin is expressed by the cell, and recovering the expressed modified immunoglobulin.

111. A method of producing a modified immunoglobulin that immunospecifically binds an antigen of an infectious disease agent, which antigen is a first member of a binding pair consisting of said first member and a second member, said modified immunoglobulin comprising a variable domain having at least one CDR containing a portion of said second member, said portion containing a binding site for said antigen and not being found naturally in the CDR, comprising growing a recombinant cell containing a nucleic acid comprising a nucleotide sequence encoding the modified immunoglobulin such that the encoded modified immunoglobulin is expressed by the cell, and recovering the expressed modified immunoglobulin.

112. A method of producing a modified immunoglobulin that immunospecifically binds a cellular receptor for an infectious disease agent, which cellular receptor is a first member of a binding pair consisting of said first member and a second member, said modified immunoglobulin comprising a variable domain having at least one CDR containing a portion of said second member, said portion containing a binding site for said cellular receptor, which binding site does not have the sequence Asn-Ala-Asn-Pro or Asn-Val-Asp-Pro and is not found naturally in the CDR, comprising growing a recombinant cell containing a nucleic acid comprising a nucleotide sequence encoding the modified immunoglobulin such that the encoded modified immunoglobulin is expressed by the cell, and recovering the expressed modified immunoglobulin.

113. A method of producing a modified immunoglobulin that immunospecifically binds a ligand, which ligand is a first member of a binding pair consisting of said first member and a second member, said modified immunoglobulin comprising a variable domain having at least one CDR containing a portion of said second member, said portion containing a binding site for said ligand and not being found naturally in the CDR, said method comprising growing a recombinant cell containing a nucleic acid comprising a nucleotide sequence encoding the modified immunoglobulin such that the encoded modified immunoglobulin is expressed by the cell, and recovering the expressed modified immunoglobulin.

114. A method of producing a modified immunoglobulin that immunospecifically binds a receptor, which receptor is a first member of a binding pair consisting of said first member and a second member, said modified immunoglobulin comprising a variable domain having at least one CDR containing a portion of said second member. said portion containing a binding site for said receptor and not being found naturally in the CDR, said method comprising growing a recombinant cell containing a nucleic acid comprising a nucleotide sequence encoding the modified immunoglobulin such that the encoded modified immunoglobulin is expressed by the cell, and recovering the expressed modified immunoglobulin.

115. A method of producing a nucleic acid encoding the modified immunoglobulin of claim 1. 13. 23 or 29 comprising:

(a) synthesizing a set of oligonucleotides, said set comprising oligonucleotides containing a portion of the nucleotide sequence that encodes the modified immunoglobulin and oligonucleotides containing a portion of the nucleotide acid sequence that is complementary to the nucleotide sequence that encodes the modified immunoglobulin, and each of said oligonucleotides having overlapping terminal sequences with another oligonucleotide of said set. except for those oligonucleotides containing the nucleotide sequences encoding the N-terminal and C-terminal portions of the modified immunoglobulin;

(b) allowing the oligonucleotides to hybridize to each other; and

(c) ligating the hybridized oligonucleotides,

such that a nucleic acid containing the nucleotide sequence encoding the modified immunoglobulin is produced.

116. A method of producing a modified immunoglobulin that immunospecifically binds a first member of a binding pair, which binding pair consists of said first member and a second member, said immunoglobulin comprising a variable domain having at least one CDR containing a portion of said second member, said portion containing a binding site for said first member and not being found naturally in the CDR, said first member being a cancer antigen, said method comprising:

(a) growing a recombinant cell containing a nucleic acid produced by the method of claim115 such that the encoded modified immunoglobulin is expressed by the cell; and

(b) recovering the expressed modified immunoglobulin.

117. A method of producing a modified immunoglobulin that immunospecifically binds a first member of a binding pair, which binding pair consists of said first member and a second member, said antibody comprising a variable domain having at least one CDR containing a portion of said second member, said portion containing a binding site for said first member and not being found naturally in the CDR, said first member being an antigen of an infectious disease agent, said method comprising:

(a) growing a recombinant cell containing a nucleic acid produced by the method of claim 115 such that the encoded modified immunoglobulin is expressed by the cell; and

(b) recovering the expressed modified immunoglobulin.

118. A method of producing a modified immunoglobulin that immunospecifically binds a first member of a binding pair, which binding pair consists of said first member and a second member, said antibody comprising a variable domain having at least one CDR containing a portion of said second member, said portion containing a binding site for said first member, which binding site does not have the sequence Asn-Ala-Asn-Pro or Asn-Val-Asp-Pro and is not found naturally in the CDR, said first member being a cellular receptor for an infectious disease agent, said method comprising:

(a) growing a recombinant cell containing a nucleic acid produced by the method of claim 115 such that the encoded modified immunoglobulin is expressed by the cell; and

(b) recovering the expressed modified immunoglobulin.

119. A method of producing a modified immunoglobulin that immunospecifically binds a first member of a binding pair, which binding pair consists of said first member and a second member, said antibody comprising a variable domain having at least one CDR containing a portion of said second member, said portion containing a binding site for said first member and not being found naturally in the CDR, said first member being a ligand, said method comprising:

(a) growing a recombinant cell containing a nucleic acid produced by the method of claim 115 such that the encoded modified immunoglobulin is expressed by the cell; and

(b) recovering the expressed modified immunoglobulin.

120. A method of producing a modified immunoglobulin that immunospecifically binds a first member of a binding pair, which binding pair consists of said first member and a second member, said antibody comprising a variable domain having at least one CDR containing a portion of said second member, said portion containing a binding site for said first member and not being found naturally in the CDR, said first member being a receptor, said method comprising:

(a) growing a recombinant cell containing a nucleic acid produced by the method of claim 115 such that the encoded modified immunoglobulin is expressed by the cell; and

(b) recovering the expressed modified immunoglobulin.

121. An isolated nucleic acid produced by the method of claim 115.

122. The isolated nucleic acid of claim 121 which is a vector.

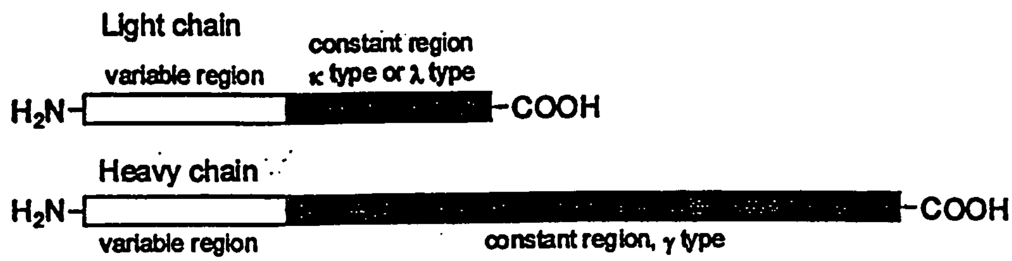

Light chain
variable region
constant region
κ type or λ type
H2N-
-COOH
Heavy chain
H2N-
-COOH
variable region
constant region, γ type

FIG. 1

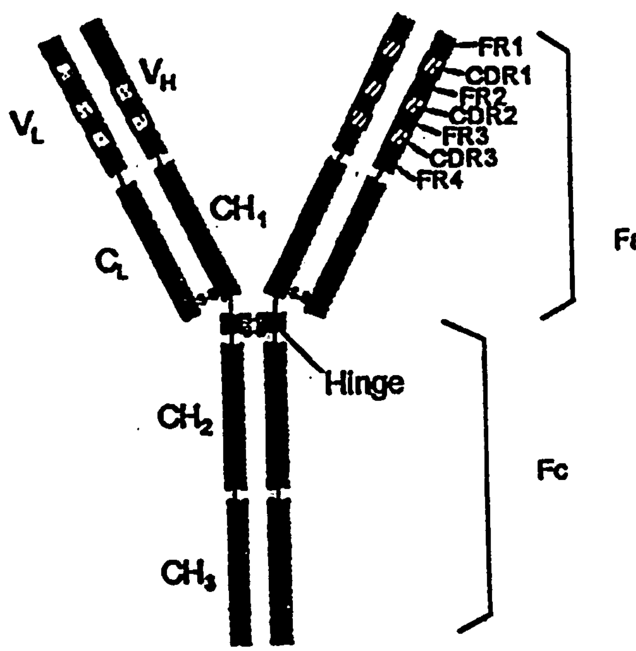


FIG. 2

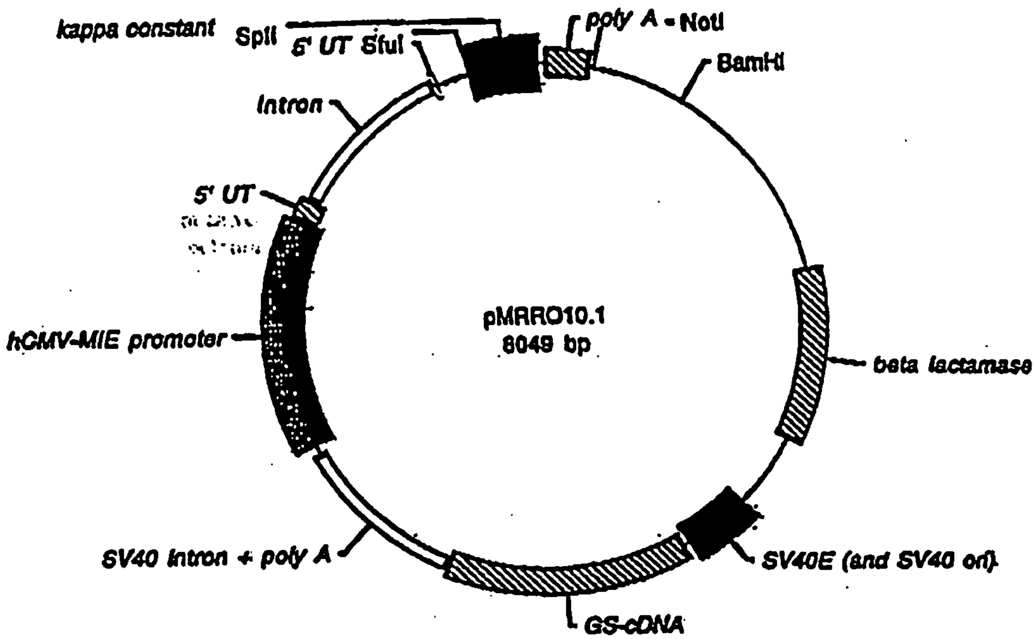

kappa constant
SplI
5' UT SfuI
poly A - NotI
BamHI
Intron
5' UT
hCMV-MIE promoter
pMRRO10.1
8049 bp
beta lactamase
SV40 intron + poly A
SV40E (and SV40 ori)
GS-cDNA

FIG. 3A

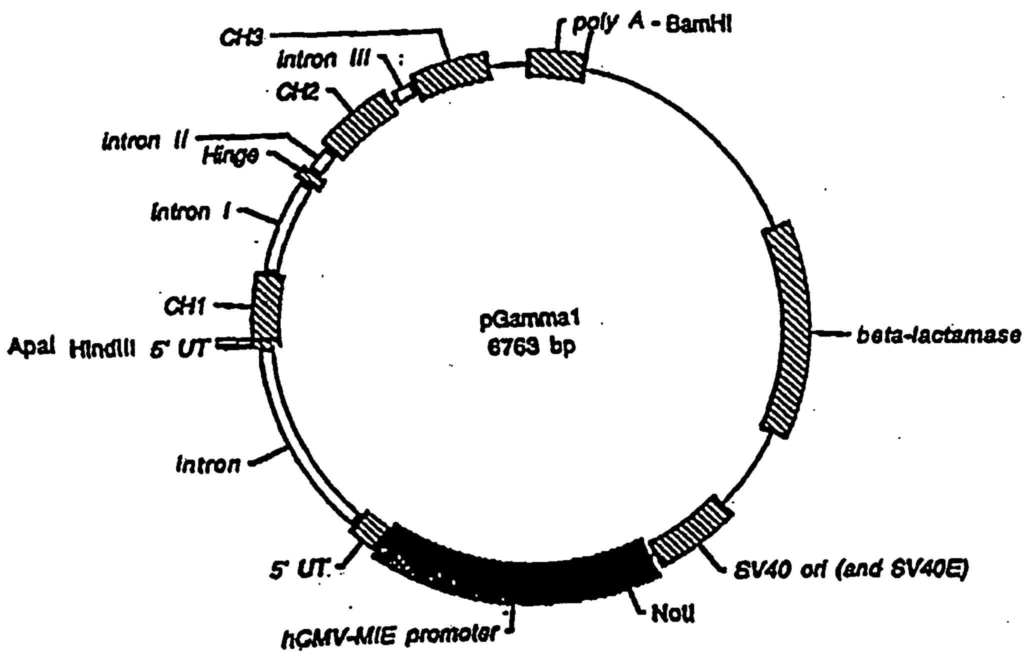

CH3
Intron III
CH2
Intron II
Hinge
Intron I
CH1
ApaI HindIII 5' UT
Intron
5' UT
hCMV-MIE promoter
NotI
SV40 ori (and SV40E)
beta-lactamase
poly A - BamHI
pGamma1
6763 bp

FIG. 3B

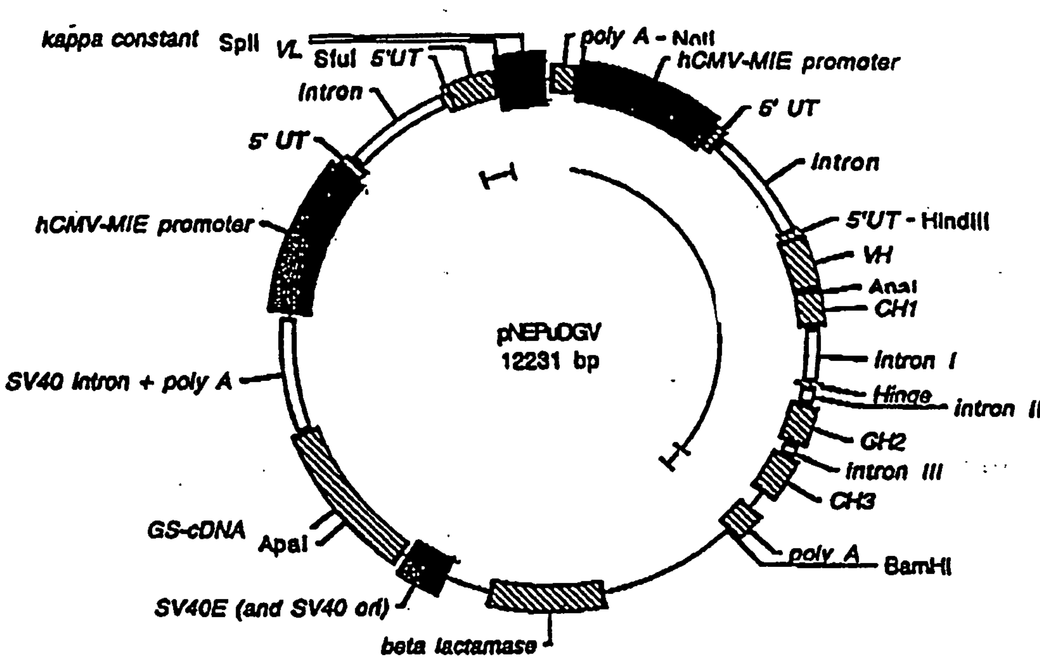

kappa constant
SplI
VL
StuI
5'UT
poly A - NotI
hCMV-MIE promoter
Intron
5' UT
5' UT
Intron
hCMV-MIE promoter
5'UT - HindIII
VH
AnaI
CH1
pNEPuDGV
12231 bp
Intron I
SV40 Intron + poly A
Hinge
Intron II
CH2
Intron III
CH3
GS-cDNA
ApaI
poly A
BamHI
SV40E (and SV40 ori)
beta lactamase

FIG. 3C

ConVL1

EcoR1
GAA TTC
6

-19 (Leader) -1
Met Ala trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC CAA AGT GCC CAA GCA
63

V_L:

1 10 20
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr
GAT ATC CAA ATG ACA CAA AGT CCT AGT AGT TTG AGT GCT AGT GTG GGA GAT CGG GTG ACA 123

21 30 40
Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro
ATC ACA TGT CGG GCT AGT CAA AGT ATC AGT AAC TGT TTG GCT TGG TAT CAA CAA AAG CCT 183

41 50 60
Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser
GGA AAG GCT CCT AAG TTG TTG ATC TAT GCT GCT AGT AGT TTG GAG AGT GGA GTG CCT AGT 243

61 70 80
Arg Phe Ser Gly Ser Gly Ser Gly Thr Arg Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
CGG TTC AGT GGA AGT GGA AGT GGA ACA CGG TTC ACC TTG ACC ATC AGT AGT TTG CAA CCT 303

81 90 100
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Leu Pro Trp Thr Phe Gly Gln
GAG GAT TTC GCT ACC TAT TAT TGT CAA CAA TAT AAC AGT TTG CCT TGG ACC TTC GGA CAA 363

101
Gly Thr Lys Val Glu Ile Lys
GGA ACC AAG GTG GAG ATC AAG GAA TTC 390
Eco R1

FIG. 4A

BKCDR1

EcoR1
GAA TTC 6

-19 (Leader) -1
Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC CAA AGT GCC CAA GCA
63

V_L:

1 10 20
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val
Thr
GAT ATC CAA ATG ACA CAA AGT CCT AGT AGT TTG AGT GCT AGT GTG GGA GAT CAA GTG
ACA . 123

21 30 40
Ile Thr Cys Arg Pro Pro Gly Phe Ser Pro Phe Arg Leu Ala Trp Tyr Gln Gln Lys
Pro
ATC ACA TGT CAA CCT CCT GGC TTC TCT CCT TTC AGG TTG GCT TGG TAT CCA CAA AAG
CCT 183

41 50 60
Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro
Ser
GGa AGG GCT CCT AAG TTG TTG ATC TAT GCT GCT AGT AGT TTG GAG AGT GGA GTG CCT
AGT 243

61 70 80
Arg Phe Ser Gly Ser Gly Ser Gly Thr Arg Phe Thr Leu Thr Ile Ser Ser Leu Gln
Pro
CGG TTC AGT GGA AGT GGA AGT GGA ACA CGG TTC ACC TTG ACC ATC AGT AGT TTG CAA
CCT 303

81 90 100
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Leu Pro Trp Thr Phe Gly
Gln
GAG GAT TTC GCT ACC TAT TAT TGT CAA CAA TAT AAC AGT TTG CCT TGG ACC TTC GGA
CAA 363

101
Gly Thr Lys Val Glu Ile Lys
GGA ACC AAG GTG GAG ATC AAG GAA TTC 390
EcoR1

FIG. 4B

BKCDR2

EcoR1
GAA TTC 6

-19 (Leader) -1
Met Ala trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC CAA AGT GCC CAA GCA
63

V_L:

1 10 20
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr
GAT ATC CAA ATG ACA CAA AGT CCT AGT AGT TTG AGT GCT AGT GTG GGA GAT CAA GTG ACA 123

21 30 40
Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro
ATC ACA TGT CGG GCT AGT CAA AGT ATC AGT AAC TAT TTG GCT TGG TAT CAA CAA AAG CCT 183

41 50 60
Gly Lys Ala Pro Lys Leu Leu Ile Tyr Pro Gly Phe Ser Pro Phe Arg Gly Val Pro Ser
GGA AAG GCT CCT AAG TTG TTG ATC TAT CCT GGC TTC TCT CCT TTC CGG GGA GTG CCT AGT 243

61 70 80
Arg Phe Ser Gly Ser Gly Ser Gly Thr Arg Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
CGG TTC AGT GGA AGT GGA AGT GGA ACA CGG TTC ACC TTG ACC ATC AGT AGT TTG CAA CCT 303

81 90 100
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Leu Pro Trp Thr Phe Gly Gln
GAG GAT TTC GCT ACC TAT TAT TGT CAA CAA TAT AAC AGT TTG CCT TGG ACC TTC GGA CAA 363

101
Gly Thr Lys Val Glu Ile Lys
GGA ACC AAG GTG GAG ATC AAG GAA TTC 390
EcoR1

FIG. 4C

BKCDR3

```
EcoR1
GAA TTC                                                     6

-19 (Leader)                                             -1
Met Ala trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC CAA AGT GCC CAA GCA
63

VL:
1                 10                  20
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val
Thr
GAT ATC CAA ATG ACA CAA AGT CCT AGT AGT TTG AGT GCT AGT GTG GGA GAT CAA GTG
ACA   123

21                30                  40
Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Asn Tyr Leu Ala Trp Tyr Gln Gln Lys
Pro
ATC ACA TGT CGG GCT AGT CAA AGT ATC AGT AAC TAT TTG GCT TGG TAT CAA CAA AAG
CCT   183

41                50                  60
Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro
Ser
GGA AAG GCT CCT AAG TTG TTG ATC TAT GCT GCT AGT AGT TTG GAG AGT GGA GTG CCT
AGT   243

61                70                  80
Arg Phe Ser Gly Ser Gly Ser Gly Thr Arg Phe Thr Leu Thr Ile Ser Ser Leu Gln
Pro
CGG TTC AGT GGA AGT GGA AGT GGA ACA CGG TTC ACC TTG ACC ATC AGT AGT TTG CAA
CCT   303

81                90                  100
Glu Asp Phe Ala Thr Tyr Tyr Cys Arg Pro Pro Gly Phe Ser Pro Phe Arg Phe Gly
Gln
GAG GAT TTC GCT ACC TAT TAT TGT AGG CCT CCT GGC TTC TCT CCT TTC AGG TTC GGA
CAA   363

101
Gly Thr Lys Val Glu Ile Lys
GGA ACC AAG GTG GAG ATC AAG GAA TTC                           390
                            EcoR1
```

FIG. 4D

ConVH1

EcoR1 6
GAA TTC

-19 (Leader) -1
Met Ala trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala
Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC
CAA AGT GCC CAA GCA 63

V_L:
1 10 20
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro
Gly Ala Ser Val Lys Val
CAG GTT CAG CTG GTG CAG TCT GGC GCT GAG GTG AAG AAG CCT
GGC GCT TCT GTG AAG GTG 123

21 30 35A 35B
40
Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Ala Ile
Ser Trp Asn Trp Val Arg Gln Ala
TCT TGC AAG GCT TCT GGC TAC ACA TTC ACA TCT TAC GCT ATA
TCT TGG AAT TGG GTG AGG CAG GCT 189

41 50 60
Pro Gly Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Gly Asn
Gly Asp Thr Asn Tyr Ala
CCT GGC CAG GGC CTG GAG TGG ATG GGC TGG ATA AAT GGA AAT
GGA GAT ACA AAT TAC GCC 249

61 70 80
Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Thr Ser
Thr Ser Thr Ala Tyr Met
CAG AAG TTC CAG GGA AGG GTG ACT ATA ACT GCT GAT ACT TCT
ACT TCT ACT GCT TAC ATG 309

81 82A 82B 82C 90
100
Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr
Cys Ala Arg Ala Pro Gly Tyr Gly Ser
GAG CTG TCT TCT CTG AGG TCT GAG GAT ACT GCT GTT TAC TAC
TGC GCT AGG GCT CCT GGC TAC GGC TCT 378

101 110
Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
GAT TAT TGG GGA CAG GGA ACA CTG GTT ACA GTT TCT TCT GAA TTC
423

FIG. 4E

BKCDR4

EcoR1 6
GAA TTC

-19 (Leader) -1
Met Ala trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC CAA AGT GCC CAA GCA
6 3

V_L:

1 10 20
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys
Val
CAG GTT CAG CTG GTG CAG TCT GGC GCT GAG GTG AAG AAG CCT GGC GCT TCT GTG AAG
GTG 123

21 30 35A 35B 40
Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Pro Gly Phe Ser Pro Phe Arg Trp Val
Arg Gln Ala
TCT TGC AAG GCT TCT GGC TAC ACA TTC ACA CCT GGC TTC TCT CCT TTC AGG TGG GTG
AGG CAG GCT 189

41 50 60
Pro Gly Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Gly Asn Gly Asp Thr Asn Tyr
Ala
CCT GGC CAG GGC CTG GAG TGG ATG GGC TGG ATA AAT GGA AAT GGA GAT ACA AAT TAC
GCC 249

61 70 80
Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr
Met
CAG AAG TTC CAG GGA AGG GTG ACT ATA ACT GCT GAT ACT TCT ACT TCT ACT GCT TAC
ATG 309

81 82A 82B 82C 90 100
Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ala Pro
Gly Tyr Gly Ser
GAG CTG TCT TCT CTG AGG TCT GAG GAT ACT GCT GTT TAC TAC TGC GCT AGG GCT CCT
GGC TAC GGC TCT 378

101 110
Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
GAT TAT TGG GGA CAG GGA ACA CTG GTT ACA GTT TCT TCT GAA TTC
423

FIG. 4F

BKCDR5

EcoR1
GAA TTC 6

-19 (Leader) -1
Met Ala trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC CAA AGT GCC CAA GCA
63

V_L:

1 10 20
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val
CAG GTT CAG CTG GTG CAG TCT GGC GCT GAG GTG AAG AAG CCT GGC GCT TCT GTG AAG GTG 123

21 30 35A 35B 40
Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Ala Ile Ser Trp Asn Trp Val Arg Gln Ala
TCT TGC AAG GCT TCT GGC TAC ACA TTC ACA TCT TAC GCT ATA TCT TGG AAT TGG GTG AGG CAG GCT 189

41 50 60
Pro Gly Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Gly Arg Pro Pro Gly Phe Ser Pro
CCT GGC CAG GGC CTG GAG TGG ATG GGC TGG ATA AAT GGA AGG CCT CCT GGC TTC TCT CCT 249

61 70 80
Phe Arg Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr Met
TTC AAG TTC CAG GGA AGG GTG ACT ATA ACT GCT GAT ACT TCT ACT TCT ACT GCT TAC ATG 309

81 82A 82B 82C 90 100
Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ala Pro Gly Tyr Gly Ser
GAG CTG TCT TCT CTG AGG TCT GAG GAT ACT GCT GTT TAC TAC TGC GCT AGG GCT CCT GGC TAC GGC TCT 378

101 110
Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
GAT TAT TGG GGA CAG GGA ACA CTG GTT ACA GTT TCT TCT GAA TTC
423

FIG. 4G

BKCDR6

EcoR1
GAA TTC
6

-19 (Leader) -1
Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser Ala Gln Ala
ATG GCT TGG GTG TGG ACC TTG CTA TTC CTG ATG GCA GCT GCC CAA AGT GCC CAA GCA
63

V_L:

1 10 20
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val
CAG GTT CAG CTG GTG CAG TCT GGC GCT GAG GTG AAG AAG CCT GGC GCT TCT GTG AAG GTG
123

21 30 35A 35B 40
Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Ala Ile Ser Trp Asn Trp Val Arg Gln Ala
TCT TGC AAG GCT TCT GGC TAC ACA TTC ACA TCT TAC GCT ATA TCT TGG AAT TGG GTG AGG CAG GCT
189

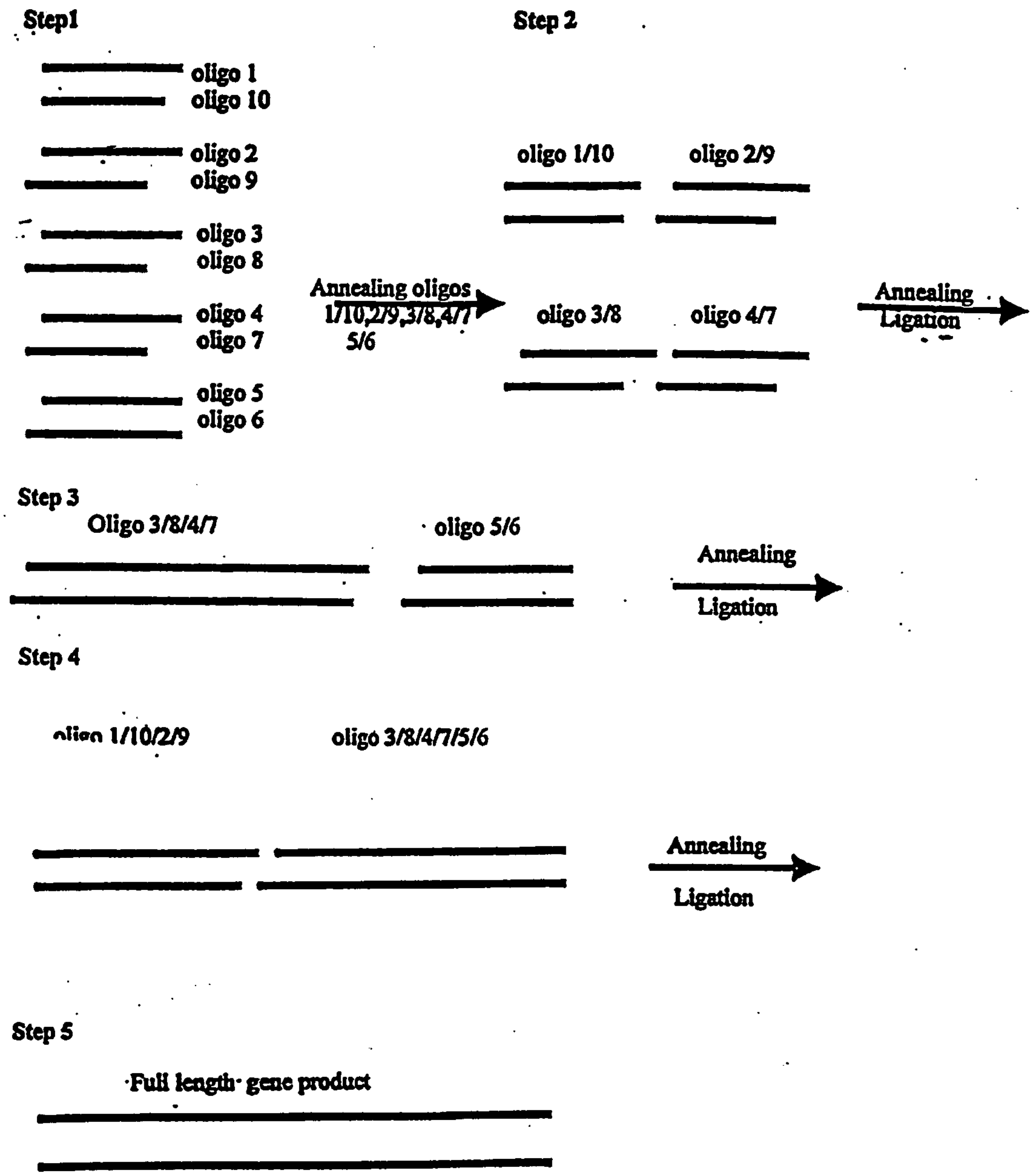
41 50 60
Pro Gly Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Gly Asn Gly Asp Thr Asn Tyr Ala
CCT GGC CAG GGC CTG GAG TGG ATG GGC TGG ATA AAT GGA AAT GGA GAT ACA AAT TAC GCC
249

61 70 80
Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr Met
CAG AAG TTC CAG GGA AGG GTG ACT ATA ACT GCT GAT ACT TCT ACT TCT ACT GCT TAC ATG
309

81 82A 82B 82C 90 100
Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Pro Pro Gly Phe Ser Pro
GAG CTG TCT TCT CTG AGG TCT GAG GAT ACT GCT GTT TAC TAC TGC GCT AGG CTT CCT GGC TTC TCT CCT
378

101 110
Phe Arg Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
TTC AGG TGG GGA CAG GGA ACA CTG GTT ACA GTT TCT TCT GAA TTC
423

FIG. 4H



Step1
oligo 1
oligo 10
oligo 2
oligo 9
oligo 3
oligo 8
oligo 4
oligo 7
oligo 5
oligo 6
Annealing oligos 1/10,2/9,3/8,4/7 5/6
Step 2
oligo 1/10
oligo 2/9
oligo 3/8
oligo 4/7
Annealing
Ligation
Step 3
Oligo 3/8/4/7
oligo 5/6
Annealing
Ligation
Step 4
oligo 1/10/2/9
oligo 3/8/4/7/5/6
Annealing
Ligation
Step 5
Full length gene product

FIG. 5

ConVL1

Leader Sequence

L1 5'GAATTCATGGCTTGGGTGTGGACCTTGCTATTCCTGATGGCAGCTGCCCAAAGTGCCC AAGCA 3'

L2 5'ACTTTGGGCAGCTGCCATCAGGAATAGCAAGGTCCACACCCAAGCCATGAATTC 3'

BKLC1 5'GATATCCAAATGACACAAAGTCCTAGTAGTTTGAGTGCTAGTGTGGGAGATCG GGTGATCACA 3'

BKLC2 5' TGTCGGGCTAGTCAAAGTATCAGTAACTATTTGGCTTGGTATCAACAAAAGCCT GGAAAGGCTCCTAAGTTGTTGATC 3'

BKLC3 5' TATGCTGCTAGTAGTTTGGAGAGTGGAGTGCCTAGTCGGTTCAGTGGA 3'

BKLC4 5' AGTGGAAGTGGAACACGGTTCACCTTGACCATCAGTAGTTTGCAACCTGAGGA TTCGCTACCTATTAT 3'

BKLC5 5' TGTCAACAATATAACAGTTTGCCTTGGACCTTCGGACAAGGAACCAAGGTGGA GATCAAGGAATTC3'

BKLC6 5' GAATTCCTTGATCTCCACCTTGGTTCCTTGTCCGAAGGTCCAAGGCAAACTGTTA TATTGTTGACAATAATAGGT3'

BKLC7 5'AGCGAAATCCTCAGGTTGCAAACTACTGATGGTCAAGGTGAACCGTGTTCCACTT CCACTTCCACTGAA3'

BKLC8 5'CCGACTAGGCACTCCACTCTCCAAACTACTAGCAGCATAGATCAACAA 3'

BKLC9 5' CTTAGGAGCCTTTCCAGGCTTTTGTTGATACCAAGCCAAATAGTTACTGATACT TTGACTAGCCCGACATGTGATTGT 3'

BKLC10 5'CACCCGATCTCCCACACTAGCACTCAAACTACTAGGACTTTGTGTCATTTGGA TATCTTGCTTGGGC3'

BKLCDR12 5'TGTCGGCCTCCTGGCTTCTCTCCTTTCAGGTTGGCTTGGTATCAACAAAAGC CTGGAAAGGCTCCTAAGTTGTTGATC 3'

BKLCDR19 5'CTTAGGAGCCTTTCCAGGCTTTTGTTGATACCAAGCCAACCTGAAAGGAGA GAAGCCAGGAGGCCGACATGTGATTGT3'

BKLCDR23 5'TATCCTGGCTTCTCTCCTTTCAGGGGAGTGCCTAGTCGGTCAGTGGA 3'

BKLCDR28 5'CCGACTAGGCACTCCCCTGAAAGGAGAGAAGCCAGGATAGATCAACAA 3'

BKLCDR35 5'TGTAGGCCTCCTGGCTTCTCTCCTTTCAGGTTCGGACAAGGAACCAAGGTGG AGATCAAG 3'

BKLCDR36 5' GAATTCCTTGATCTCCACCTTGGTTCCTTGTCCGAACCTGAAAGGAGAGAA GCCAGGAGGCCTACAATAATAGGT 3'

FIG. 6A

ConVH1

BKHC1 5'GAATTCATGGCTTGGGTGTGGACCTTGCTATTCCTGATGGCAGCTGCCCAAAGTG CCCAAGCACAGATCCAGTTGGTGCAGTCTG 3'

BKHC2 5'GCGCTGAGGTGAAGAAGCCTGGCGCTTCTGTGAAGGTGTCTTGCAAGGCTTCT GGCTACATTCACATCTTACGCTATATCTTG 3'

BKHC3 5'GAATTGGGTGAGGCAGGCTCCTGGCCAGGGCCTGGAGTGGATGGGCTGGATAAAT GGAAATGGAGATACAATTACGCCCAGAAG 3'

BKHC4 5'TTCCAGGGAAGGGTTACTATAACTGCTGATACTTCTACTTCTACTGCTTACATGG AGCTGTCTTCTCTGAGGTCTGAGGATACT 3'

BKHC5 5'GCTGTTTACTACTGCGCTAGGGCTCCTGGCTACGGCTCTGATTATTGGGGACA GGGAACACTGGTTACAGTTTCTTTCTGAATTC 3'

BKHC6 5'GAATTCAGAAGAAACTGTAACCAGTGTTCCCTGTCCCCAATAATCAGAGCCGTA GCCAGGAGCC 3'

BKHC7 5' CTAGCGCAGTAGTAAACAGCAGTATCCTCAGACCTCAGAGAAGACAGCTCCAT GTAAGCAGTAGAAGTAGAAGTATCAGCAGTT 3'

BKHC8 5'ATAGTAACCCTTCCCTGGAACTTCTGGGCGTAATTTGTATCTCCATTTCCATTT ATCCAGCCCATCCACTCCAGGCCCTGGCCAG 3'

BKHC9 5'GAGCCTGCCTCACCCAATTCCAAGATATAGCGTAAGATGTGAATGTGTAGCCA GAAGCCTTGCAAGACACCTTCACAGAAGCGCC 3'

BKHC10 5'AGGCTTCTTCACCTCAGCGCCAGACTGCACCAGCTGAACCTGTGCTTGGGCACT TTGGGCAGCTGCCATCAGGAATAGCAAGGTCCACACCCAAGCCATGAATTC 3'

BKHCDR42 5'GCGCTGAGGTGAAGAAGCCTGGCGCTTCTGTGAAGGTGTCTTGCAAGGC TTCTGGCTACACATTCACA 3'

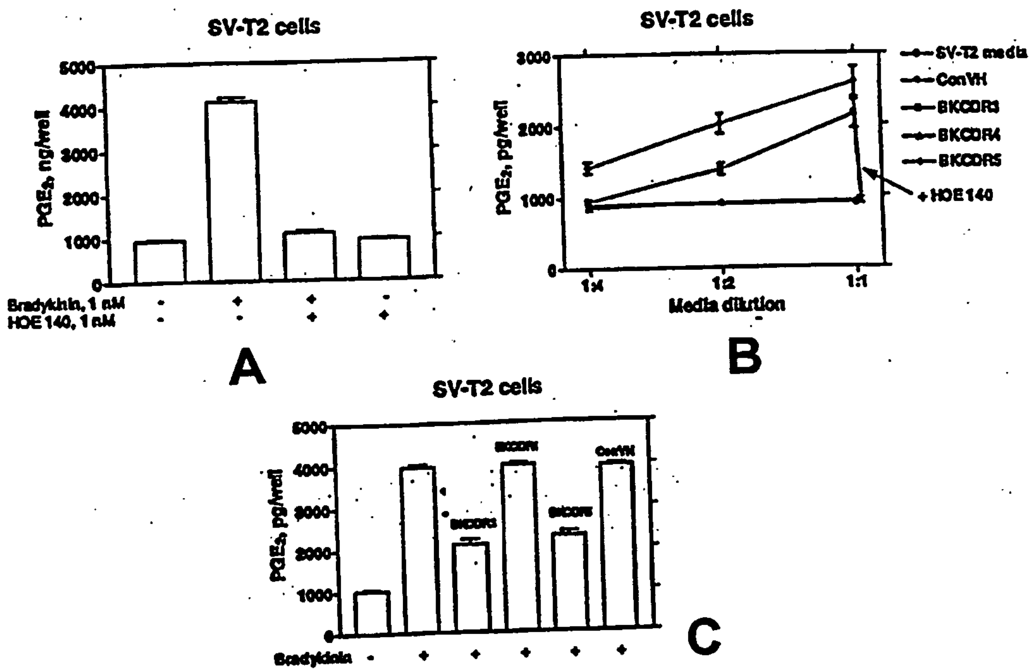
BKHDR43 5'CAGGTGGGTGAGGCAGGCTCCTGGCCAGGGCCTGGAGTGGATGGGCTGGAT AAATGGAGATACAAATTACGCCCAGAAG 3'

BKHDR49 5'GAGCCTGCCTCACCCACCTGAAAGGAGAGAAGCCAGGTGTGAATGTGTA GCCAGAAGCCTTGCAAGACACCTTCACAGAAGCGCC 3'

BKHDR53 5'GAATTGGGTGAGGCAGGCTCCTGGCCAGGGCCTGGAGTGGATGGGCTGGATA AATGGAAGGCCTCCTGGCTTCTCTCCTTTCAGG 3'

BKHDR58 5'ATAGTAACCCTTCCCTGGAACCTGAAAGGAGAGAAGCCAGGAGGCCTTC CATTTATCCAGCCCATCCACTCCAGGCCCTGGCCAG 3'

FIG. 6B


SV-T2 cells
PGE₂, ng/well
Bradykinin, 1 nM
HOE 140, 1 nM
A
SV-T2 cells
PGE₂, pg/well
Media dilution
SV-T2 media
ConVH
BKCDR3
BKCDR4
BKCDR5
+HOE140
B
SV-T2 cells
PGE₂, pg/well
Bradykinin
C

FIGS. 7A-C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/24302

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 39/395; C12N 5/12, 15/13; C07K 16/42, 16/08, 16/30
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,653,977 A (SALEH) 05 August 1997, see entire document.	1-4, 39-67, 71-95, 99, 104, 106, 109-110, 115-117, 119-122
Y	RUDIKOFF et al. Functional Antibody Lacking a Variable-region Disulfide Bridge. Proc Natl Acad Sci USA. October 1986, Vol. 83, pages 7875-7878, see entire document.	63-66

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
01 APRIL 1999	16 APR 1999

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Julie Reeves Laurence Fa
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/24302

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	BILLETTA et al. Immunogenicity of an Engineered Internal Image Antibody. Proc Natl Acad Sci USA. June 1991, Vol. 88, 4713-4717, see entire document.	29-30, 70, 102-103, 113-114 and claims 39, 43, 46-48, 51, 72-73, 78-94, 115, 117, 119-122, wherein they depend upon claims 29, 30 or 70 --- 1-4, 13-15, 20, 38, 40-42, 44-45, 49-50, 52-68, 71, 75, 77, 95-100, 102-107, 109-111, 116, 118
X - Y	US 5,476,784 A (RICE et al) 19 December 1995, see entire document.	13-15, 20, 39, 43, 46-56, 68, 72-75, 78-94, 96, 100, 105, 107, 109, 111, 115, 117, 119-122 --- 1-4, 29-30, 70
Y	WO 94/11509 A2 (CANCER RESEARCH FUND OF CONTRA COSTRA) 26 May 1994, see entire document.	1-4, 13-15, 20, 23-25, 29-30, 38-68, 70-100, 102-107, 109-111, 113-117, 119-122
Y	TAUB et al. Peptide Sequence from the Hypervariable regions of Two monoclonal anti-idiotypic antibodies against the Thyrotropin (TSH) receptor are similar to TSH and inhibit TSH-increased cAMP production in FRTL-5 thyroid cells. The Journal of Biological Chemistry. 25 March 1992, Vol. 267, No. 9, pp 5977-5984, see entire article.	29, 30, 49, 52

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/24302

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	ROSEL et al. Adhesive Functions of Antibodies Antigenized with the Arg Gly Asp (RGD) Epitope. FASEB Journal. 1992,. Vol 6, No. 4, page A1400, Abstract 2691, see entire document.	29-30, 70, 97-98, 102-103 and claims 39, 42, 46-48, 51, 72-73 wherein they depend upon claims 30 or 70 --- 113-114 and claims 40-41, 43-45, 49-50, 52-56, 59-66, 71, 74-94, 109, 115, 117, 119-122 wherein they depend upon claims 29, 30 or 70.
Y	BRUMEANU et al. Efficient Loading of Identical Viral peptide onto Class II Molecules by Antigenized immunoglobulin and Influenza Virus. Journal Experimental Medicine. November 1993, Vol. 178, pages 1795-1799, see entire document.	13-14, 29-30, 39-66, 68, 70-94, 96-98, 100, 102-103, 105, 107, 109-111, 113-115, 117, 119-122.
X - Y	US 5,508,386 A (ZANETTI et al) 16 April 1996, see entire document.	29-30, 70, 102-103, 113-114 and claims 39, 43, 46-48, 51, 72-73, 78-94, 115, 117, 119-122 wherein they depend upon claims 29, 30 or 70 --- 1-4, 13-15, 20, 38, 40-42, 44-45, 49-50, 52-68, 71, 74-77, 95-100, 102-107, 109-111, 113-114, 116
Y	US 5,624,904 A (KRIEGER et al) 29 April 1997, see entire document.	13-15, 20, 39-66, 68, 71-94, 96, 100, 105, 107, 109, 111, 115, 117, 119-122.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/24302

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CORTHESY et al. A Pathogen-specific Epitope Inserted into Recombinant Secretory Immunoglobulin A is Immunogenic by the Oral Route. Journal Biological Chemistry. 27 December 1996, Vol. 271, No. 52, pages 33670-33677, see entire document.	13-15, 20, and 39-66 wherein they depend upon claims 13 or 14.
X	LANZA et al. Analysis of syncytial-inhibiting anti-CD4 antibodies elicited by antigenized antibodies expressing oligopeptides of human CD4 in a hypervariable loop. IXth International Conference on AIDS and IVth STD World Congress. 01 September 1993, page 245, Abstract PO-A28-0663, see entire document.	13-14, 29-30, 39, 42, 46-48, 51, 57-58, 68, 70, 72, 73 wherein they depend upon claims 13, 14, 29, 30 or 70.
X - Y	LANZA et al. Use of Antigenized Antibodies Containing CD4 sequences to generate antibodies able to inhibit syncytial formation. FASEB Journal. 1992, Vol. 6, No. 4, page A1400, Abstract 2690, see entire document.	13-14, 29-30, 68, 70, 92 and claims 39, 42, 46-48, 51, 57-58, 72-73 wherein they depend upon claims 14, 30, 68 or 70 --- 38, 96-98, 100, 102-103, 105, 107, 111, 113-114 and claims 40-41, 43-45, 49, 50, 52-56, 59-66, 71, 74-94, 109-115, 117, 119-122 wherein they depend upon claims 13, 14, 29, 30 68 or 70.
Y	BONA et al. Immunogenicity of microbial peptides grafted in self Immunoglobulin Molecules. Cellular and Molecular Biology, 1994, Vol. 40 (Supplement I) pages 21-30, see entire document.	1-4, 13-15, 20, 29-30, 38-68, 70-100, 102-107, 109-111, 113-117, 119-122

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Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-4, 13-15, 20, 23-25, 29-30, 38, 39-68, 70-100, 102-107, 109-111, 113-117, 119-122

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

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A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

530/387.3, 387.7, 389.7, 388.3, 388.4, 388.6, 387.2; 424/131.1, 133.1, 138.1, 155.1, 151.1, 150.1, 147.1; 435/327, 328, 330, 339, 342, 69.6

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

530/387.3, 387.7, 389.7, 388.3, 388.4, 388.6, 387.2; 424/131.1, 133.1, 138.1, 155.1, 151.1, 150.1, 147.1; 435/327, 328, 330, 339, 342, 69.6

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, STN, Medline, Biosis, Scisearch
search terms: antibody, immunoglobulin, CDRs, complementarity determining regions, variable domain, cancer, infectious agent, vaccine

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-4, 67, 95, 99, 110, 116 and claims 39-66, 71-94, 109, 115, 121-122, wherein claims 39-66, 71-94, 109, 115, 121-122 depend upon any of claims 1-4, 67, 95, 99, 110 or 116, drawn to a modified immunoglobulin that binds to a cancer antigen, a molecule comprising a variable domain that binds to a cancer antigen, a method for identifying or measuring or detecting cancer antigen using a modified immunoglobulin that binds a cancer antigen, a kit for detecting a cancer antigen, a method of producing the modified immunoglobulin or a method of producing the nucleic acid encoding the modified immunoglobulin.

Groups IIa-IIf, claim(s) 1-3, 5, 6, 67, 95, 99, 110, 116 and claims 39-66, 71-94, 109, 115, 121-122, wherein claims 39-66, 71-94, 109, 115, 121-122 depend upon any of claims 1-3, 67, 95, 99, 110 or 116, drawn to a modified immunoglobulin that binds to a cancer antigen, a molecule comprising a variable domain that binds to a cancer antigen, a method for identifying or measuring or detecting cancer antigen using a modified immunoglobulin that binds a cancer antigen, a kit for detecting a cancer antigen, a method of producing the modified immunoglobulin or a method of producing the nucleic acid encoding the modified immunoglobulin.

Group III, claim(s) 1-3, 7, 67, 95, 99, 110, 116 and claims 39-66, 71-94, 109, 115, 121-122, wherein claims 39-66, 71-94, 109, 115, 121-122 depend upon any of claims 1-3, 67, 95, 99, 110 or 116, drawn to a modified immunoglobulin that binds to a cancer antigen, a molecule comprising a variable domain that binds to a cancer antigen, a method for identifying or measuring or detecting cancer antigen using a modified immunoglobulin that binds a cancer antigen, a kit for detecting a cancer antigen, a method of producing the modified immunoglobulin or a method of producing the nucleic acid encoding the modified immunoglobulin.

Group IVa-IVf, claim(s) 1-3, 8, 9, 67, 95, 99, 110, 116 and claims 39-66, 71-94, 109, 115, 121-122, wherein claims 39-66, 71-94, 109, 115, 121-122 depend upon any of claims 1-3, 67, 95, 99, 110 or 116, drawn to a modified immunoglobulin that binds to a cancer antigen, a molecule comprising a variable domain that binds to a cancer antigen, a method for identifying or measuring or detecting cancer antigen using a modified immunoglobulin that binds a cancer antigen, a kit for detecting a cancer antigen, a method of producing the modified immunoglobulin or a method of producing the nucleic acid encoding the modified immunoglobulin.

Group Va-Ve, claim(s) 1-3, 8, 10, 67, 95, 99, 110, 116 and claims 39-66, 71-94, 109, 115, 121-122, wherein claims 39-66, 71-94, 109, 115, 121-122 depend upon any of claims 1-3, 67, 95, 99, 110 or 116, drawn to a modified immunoglobulin that binds to a cancer antigen, a molecule comprising a variable domain that binds to a cancer antigen, a method for identifying or measuring or detecting cancer antigen using a modified immunoglobulin that binds a cancer antigen, a kit for detecting a cancer antigen, a method of producing the modified immunoglobulin or a method of producing the nucleic acid encoding the modified immunoglobulin.

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Group VIa-VIk, claim(s) 1-3, 11, 67, 95, 99, 110, 116 and claims 39-66, 71-94, 109, 115, 121-122, wherein claims 39-66, 71-94, 109, 115, 121-122 depend upon any of claims 1-3, 67, 95, 99, 110 or 116, drawn to a modified immunoglobulin that binds to a cancer antigen, a molecule comprising a variable domain that binds to a cancer antigen, a method for identifying or measuring or detecting cancer antigen using a modified immunoglobulin that binds a cancer antigen, a kit for detecting a cancer antigen, a method of producing the modified immunoglobulin or a method of producing the nucleic acid encoding the modified immunoglobulin.

Group VII(1)-VII(69), claim(s) 1-3, 12, 67, 95, 99, 110, 116 and claims 39-66, 71-94, 109, 115, 121-122, wherein claims 39-66, 71-94, 109, 115, 121-122 depend upon any of claims 1-3, 67, 95, 99, 110 or 116, drawn to a modified immunoglobulin that binds to a cancer antigen, a molecule comprising a variable domain that binds to a cancer antigen, a method for identifying or measuring or detecting cancer antigen using a modified immunoglobulin that binds a cancer antigen, a kit for detecting a cancer antigen, a method of producing the modified immunoglobulin or a method of producing the nucleic acid encoding the modified immunoglobulin.

Group VIII(1)-VIII(12), claim(s) 13-15, 20, 68,69, 111, 117 and claims 39-66, 71-94, 109, 115, 121-122, wherein claims 39-66, 71-94, 109, 115, 121-122 depend upon any of claims 13, 14, 67, 111, 117 drawn to infectious agent ligand, and a molecule comprising a variable domain that binds to an infectious agent antigen.

Group IX(1)-IX(54), claim(s) 13, 14, 16, 21, 68, 69, 111, 117 and claims 39-66, 71-94, 109, 115, 121-122, wherein claims 39-66, 71-94, 109, 115, 121-122 depend upon any of claims 13, 14, 67, 111, 117 drawn to infectious agent ligand, and a molecule comprising a variable domain that binds to an infectious agent antigen.

Group X(1)-X(6), claim(s) 13, 14, 17, 22, 68, 69, 111, 117 and claims 39-66, 71-94, 109, 115, 121-122, wherein claims 39-66, 71-94, 109, 115, 121-122 depend upon any of claims 13, 14, 67, 111, 117 drawn to infectious agent ligand, and a molecule comprising a variable domain that binds to an infectious agent antigen.

Group XI(1)-XI(6), claim(s) 13, 14, 18, 68, 69, 111, 117 and claims 39-66, 71-94, 109, 115, 121-122, wherein claims 39-66, 71-94, 109, 115, 121-122 depend upon any of claims 13, 14, 67, 111, 117 drawn to infectious agent ligand, and a molecule comprising a variable domain that binds to an infectious agent antigen.

Group XII(1)-XII(43), claim(s) 13, 14, 19, 68, 69, 111, 117 and claims 39-66, 71-94, 109, 115, 121-122, wherein claims 39-66, 71-94, 109, 115, 121-122 depend upon any of claims 13, 14, 67, 111, 117 drawn to infectious agent ligand, and a molecule comprising a variable domain that binds to an infectious agent antigen.

Group XIII, claim(s) 23-25, 101, 109, 112, 115, 118, 121-122 and claims 39-66, 71-94, 109, 115, 121-122, wherein claims 39-66, 71-94, 109, 115, 121-122 depend upon any of claims 23-25 101, 109, 112, 115, 118, 121-122, drawn to a modified immunoglobulin which binds to a cellular receptor for an infectious agent.

Group XIV, claim(s) 23, 24, 26, 101, 109, 112, 115, 118, 121-122 and claims 39-66, 71-94, 109, 115, 121-122, wherein claims 39-66, 71-94, 109, 115, 121-122 depend upon any of claims 23, 24, 101, 109, 112, 115, 118, 121-122, drawn to a modified immunoglobulin which binds to a cellular receptor for an infectious agent.

Group XV, claim(s) 23, 24, 27, 101, 109, 112, 115, 118, 121-122 and claims 39-66, 71-94, 109, 115, 121-122, wherein claims 39-66, 71-94, 109, 115, 121-122 depend upon any of claims 23, 24, 101, 109, 112, 115, 118, 121-122, drawn to a modified immunoglobulin which binds to a cellular receptor for an infectious agent.

Group XVI(1)-XVI(76), claim(s) 23, 24, 28, 101, 109, 112, 115, 118, 121-122 and claims 39-66, 71-94, 109, 115, 121-122, wherein claims 39-66, 71-94, 109, 115, 121-122 depend upon any of claims 23, 24, 101, 109, 112, 115, 118, 121-122, drawn to a modified immunoglobulin which binds to a cellular receptor for an infectious agent.

Group XVII(1)-XVII(26), claim(s) 29, 30, 38, 70, and claims 39-66, 71-94, 109, 115, 121-122, wherein claims 39-66, 71-94, 109, 115, 121-122 depend upon any of claims 29, 70, drawn to a modified immunoglobulin that binds to a modified immunoglobulin that binds to a ligand receptor binding pair and, a molecule comprising a variable domain that binds to a modified immunoglobulin that binds to a ligand receptor binding pair.

Group XVIII(1)-XVIII(16), claim(s) 29, 31, 37, 70, and claims 39-66, 71-94, 109, 115, 121-122, wherein claims 39-66, 71-94, 109, 115, 121-122 depend upon any of claims 29, 70, drawn to a modified immunoglobulin that binds to a modified immunoglobulin that binds to a ligand receptor binding pair and, a molecule comprising a variable domain that

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binds to a modified immunoglobulin that binds to a ligand receptor binding pair.

Group XIX, claim(s) 29, 32, 70, and claims 39-66, 71-94, 109, 115, 121-122, wherein claims 39-66, 71-94, 109, 115, 121-122 depend upon any of claims 29, 70, drawn to a modified immunoglobulin that binds to a modified immunoglobulin that binds to a ligand receptor binding pair and, a molecule comprising a variable domain that binds to a modified immunoglobulin that binds to a ligand receptor binding pair.

Group XX, claim(s) 29, 33, 70, and claims 39-66, 71-94, 109, 115, 121-122, wherein claims 39-66, 71-94, 109, 115, 121-122 depend upon any of claims 29, 70, drawn to a modified immunoglobulin that binds to a modified immunoglobulin that binds to a ligand receptor binding pair and, a molecule comprising a variable domain that binds to a modified immunoglobulin that binds to a ligand receptor binding pair.

Group XXI, claim(s) 29, 34, 70, and claims 39-66, 71-94, 109, 115, 121-122, wherein claims 39-66, 71-94, 109, 115, 121-122 depend upon any of claims 29, 70, drawn to a modified immunoglobulin that binds to a modified immunoglobulin that binds to a ligand receptor binding pair and, a molecule comprising a variable domain that binds to a modified immunoglobulin that binds to a ligand receptor binding pair.

Group XXII, claim(s) 29, 35, 36, 70, and claims 39-66, 71-94, 109, 115, 121-122, wherein claims 39-66, 71-94, 109, 115, 121-122 depend upon any of claims 29, 70, drawn to a modified immunoglobulin that binds to a modified immunoglobulin that binds to a ligand receptor binding pair and, a molecule comprising a variable domain that binds to a modified immunoglobulin that binds to a ligand receptor binding pair.

Group XXIII, claim(s) 29, 70, and claims 39-66, 71-94, 109, 115, 121-122, wherein claims 39-66, 71-94, 109, 115, 121-122 depend upon any of claims 29, 70, drawn to a modified immunoglobulin that binds to a modified immunoglobulin that binds to a ligand receptor binding pair and, a molecule comprising a variable domain that binds to a modified immunoglobulin that binds to a ligand receptor binding pair.

Group XXIV, claim(s) 96 and 100, drawn to a method of detecting, identifying or measuring an infectious agent.

Group XXV, claim(s) 97 and 102, drawn to a method of detecting, identifying or measuring a ligand.

Group XXVI, claim(s) 98 and 103, drawn to a method of detecting, identifying or measuring a receptor.

Group XXVII, claim(s) 104, drawn to a method of diagnosing or screening for cancer.

Group XXVIII, claim(s) 105, drawn to a method of diagnosing or screening for an infectious agent.

Group XXIX, claim(s) 106, drawn to a method of treating or preventing cancer.

Group XXX, claim(s) 107, drawn to a method of treating or preventing infection.

Group XXXI, claims 113 and 119 drawn to a method of making a modified immunoglobulin which binds to a ligand.

Group XXXII, claims 114 and 120, drawn to a method of making a modified immunoglobulin which binds to a receptor.

It is noted that claims 6, 9, 10, 15, 23-28, 101, 108, 109, 112, 115, 121-122 contain sequences. As of 2/22/99, a computer readable form of the sequence listing has not been entered for this application. Because the sequences are necessary to search claims 6, 9, 10, 15, 23-28, 101, 108, 109, 112, 115, 121-122, these claims will only be examined if a computer readable form of the sequence listing has been submitted and entered.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:

For Group IIa-IIf each of the peptides of claim 6 are viewed as a distinct species as there is no apparent common core structure.

For Group IVa-IVf the peptides of claim 9 are viewed as a distinct species as there is no apparent common core structure.

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For Group Va-Ve the peptides of claim 10 are viewed as a distinct species as there is no apparent common core structure.

For Group VIa-VIk the various cancers of claim 11 do not share common antigens.

For Group VII(1)-VII(69) the antigens of claim 12 are viewed as a distinct species as there is no apparent common core structure.

For Groups VIII(1) through XII(43) claims 18-22, one of the several dozen infectious disease antigens listed must elected since there is no common core amongst them.

For Group XVI(1)-XVI(76) claim 28, one of the several dozen cellular receptors listed must elected as they lack a common core.

For Group XVII(1)-XVII(26) claim 38, one of the twenty-six receptors listed must elected as they lack a common core.

For Group XVIII(1)-XVIII(16) claim 37, one of the sixteen ligands must be elected as they lack a common core.

The inventions listed as Groups I-XII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Group I recites the technical feature of a modified immunoglobulin that binds to a cancer antigen, a molecule comprising a variable domain that binds to a cancer antigen, a method for identifying or measuring or detecting cancer antigen using a modified immunoglobulin that binds a cancer antigen, a kit for detecting a cancer antigen, a method of producing the modified immunoglobulin or a method of producing the nucleic acid encoding the modified immunoglobulin.

However, ZANETTI et al. (US 5,508,386) teaches the production of antibodies containing the NANP tetrapeptide within the third CDR. Given this teaching the invention of Group I lacks an inventive step as one of ordinary skill in the art would be motivated to employ the methodology of Zanetti et al. to develop chimeric antibodies for any antigen of interest and more particularly for a cancer antigen.

Therefore, within the meaning of PCT Rules 13.1 and 13.2 no special technical feature links the invention of Group I with Groups IIa through XXXII because the technical feature of Group I is not a contribution over the prior art.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: The amino acid sequences listed in claims 6, 9, 10, 23 and 36 all contain the special technical feature of their amino acid sequence, which imparts a unique structure and function and which requires a different search.

For claim 11, each of the different types of cancer has a special technical feature in its etiology, prognosis and pathology.

For claim 12, each of the different cancer antigens has a special technical feature of its immunogenicity.

For claims 18-22, each of the infectious disease antigens listed has a special technical feature of its immunogenicity, infectivity, pathology and etiology.

For claim 28, 37 and 38, each of the cellular receptors or ligands listed has a special technical feature of its distinct structure and function, cellular localization, tissue distribution, etc.